

## Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 3. Discovery of a Potent, 2-Nonaryl, Highly Selective $ET_A$ Antagonist (A-216546)

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Previously we have reported the discovery of ABT-627 (**1**, A-147627, active enantiomer of A-127722), a 2,4-diaryl substituted pyrrolidine-3-carboxylic acid based endothelin receptor- $A$  antagonist. This compound binds to the  $ET_A$  receptor with an affinity ( $K_i$ ) of 0.034 nM and with a 2000-fold selectivity for the  $ET_A$  receptor versus the  $ET_B$  receptor. We have expanded our structure-activity studies in this series, in an attempt to further increase the  $ET_A$  selectivity. When the *p*-anisyl group of **1** was replaced by an *n*-pentyl group, the resultant antagonist **3** exhibited substantially increased  $ET_B/ET_A$  activity ratio, but a decreased  $ET_A$  affinity. Structure-activity studies revealed that substitution and geometry of this alkyl group, and substitution on the benzodioxolyl ring, are important in optimizing this series of highly  $ET_A$  selective antagonists. In particular, the combination of a (*E*)-2,2-dimethyl-3-pentenyl group and a 7-methoxy-1,3-benzodioxol-5-yl group provided hydrophobic compound **10b** with subnanomolar affinity for human  $ET_A$  receptor subtype and with an  $ET_B/ET_A$  activity ratio of over 130000. Meanwhile, synthetic efforts en route to olefinic compounds led to the discovery that 2-pyridylethyl (**9a**) and 2-(2-oxopyrrolidinyl)ethyl (**9u**) replacement of the *p*-anisyl group of **1** yielded very hydrophilic  $ET_A$  antagonists with potency and selectivity equal to those of **10b**. On the basis of overall superior affinity, high selectivity for the  $ET_A$  receptor ( $K_i$ , 0.46 nM for  $ET_A$  and 13000 nM for  $ET_B$ ), and good oral bioavailability (48% in rats), A-216546 (**10a**) was selected as a potential clinical backup for **1**.

### Introduction

The endothelins (ET-1, ET-2 and ET-3), 21-residue amino acid peptides discovered in 1988,<sup>1,2</sup> are potent, long-acting constrictors of vascular smooth muscle and are also potent mitogens.<sup>3</sup> ET-1, made primarily by endothelial cells, is thought to act as an autocrine/paracrine mediator in the regulation of vascular functions through modulation of tone, cell proliferation, vascular permeability, and hormone production.<sup>4-7</sup>

The biological effects of the ETs are mediated by G-protein-linked receptors. Two subtypes of human endothelin receptors ( $ET_A$  and  $ET_B$ ) have been cloned and are approximately 55% homologous.<sup>8,9</sup> The  $ET_A$  receptor has greater affinity for ET-1 than ET-3 and is expressed in vascular smooth muscle cells. Binding of ET-1 to  $ET_A$  receptor mediates the vasoconstrictive and mitogenic effects of ET-1 both in vitro and in vivo.<sup>3-7</sup> The  $ET_B$  receptor has equal affinity for ET-1 and ET-3 and is expressed in endothelial cells. Binding of ET-1 or ET-3 to the  $ET_B$  receptor may attenuate the vasoconstrictive effects of local ET-1 by mediating production of nitric oxide<sup>10</sup> and by clearing ET-1 from the circulation.<sup>11</sup>

Endothelin-1 has been implicated as a contributing factor in many diseases.<sup>3-7</sup> In animal models and

human studies, ET receptor antagonists have demonstrated clear benefit in acute myocardial infarction,<sup>12</sup> congestive heart failure,<sup>13</sup> pulmonary hypertension,<sup>14</sup> cerebral vasospasm,<sup>15</sup> renal failure,<sup>16</sup> and restenosis.<sup>17</sup> It is apparent from these studies that  $ET_A$  receptors mediate most of the actions of ET-1 associated with these pathological conditions, while the  $ET_B$  receptor may mediate some beneficial effects. These findings suggest that a selective  $ET_A$  receptor antagonist would be useful as a therapeutic agent for chronic treatment of the aforementioned pathological conditions.

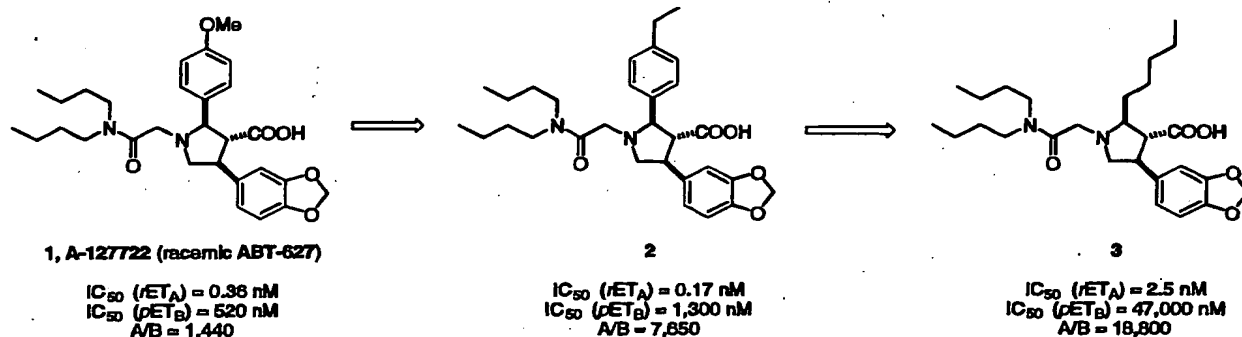
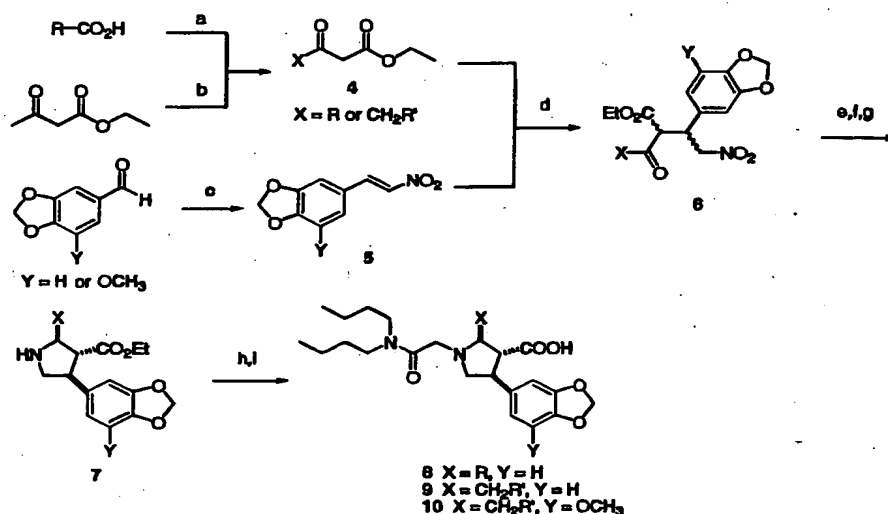
Recently, our laboratories have disclosed the design and synthesis of a novel series of 1,2,4-trisubstituted pyrrolidine-3-carboxylic acid based  $ET_A$  receptor selective antagonists, exemplified by ABT-627 (**1**, A-147627, active enantiomer of A-127722).<sup>18</sup> This compound competitively inhibits [<sup>125</sup>I]ET-1 binding to cloned human  $ET_A$  and  $ET_B$  receptors with  $K_i$  values of 0.034 and 63.3 nM, respectively. In our efforts to search for a follow-up compound possessing even greater selectivity for the  $ET_A$  receptor, the contributions to binding affinity or receptor subtype selectivity conferred by the *p*-anisyl group of **1** were investigated. When the *p*-anisyl substituent of A-127722 was changed to a *p*-ethylphenyl group (**2**), a decrease of  $ET_B$  affinity was observed with a slight increase of  $ET_A$  affinity (Scheme 1). Intrigued by this observation, we experimented with aliphatic groups and quickly discovered that the corresponding *n*-pentyl group (**3**) further improved upon the  $ET_B/ET_A$  activity ratio of **2**, though **3** exhibited decreased affinity

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Scheme 1. Rationale for Developing the Alkyl-Based Highly ET<sub>A</sub> Selective AntagonistsScheme 2<sup>a</sup>

<sup>a</sup> (a) CDI, THF, then magnesium monoethylmalonate; (b) NaH, then *n*-BuLi, then R'-Br, THF; (c) MeNO<sub>2</sub>, NH<sub>4</sub>OAc, AcOH-EtOH, reflux; (d) cat. *t*-BuOK, THF; (e) H<sub>2</sub>, Raney Ni, EtOAc; (f) NaBH<sub>3</sub>CN, concentrated HCl, THF-EtOH, pH 5; (g) DBU, CH<sub>3</sub>CN, reflux; (h) *n*-Bu<sub>2</sub>NCOCH<sub>2</sub>Br, *i*-Pr<sub>2</sub>NEt, CH<sub>3</sub>CN; (i) 6 N aqueous NaOH, EtOH.

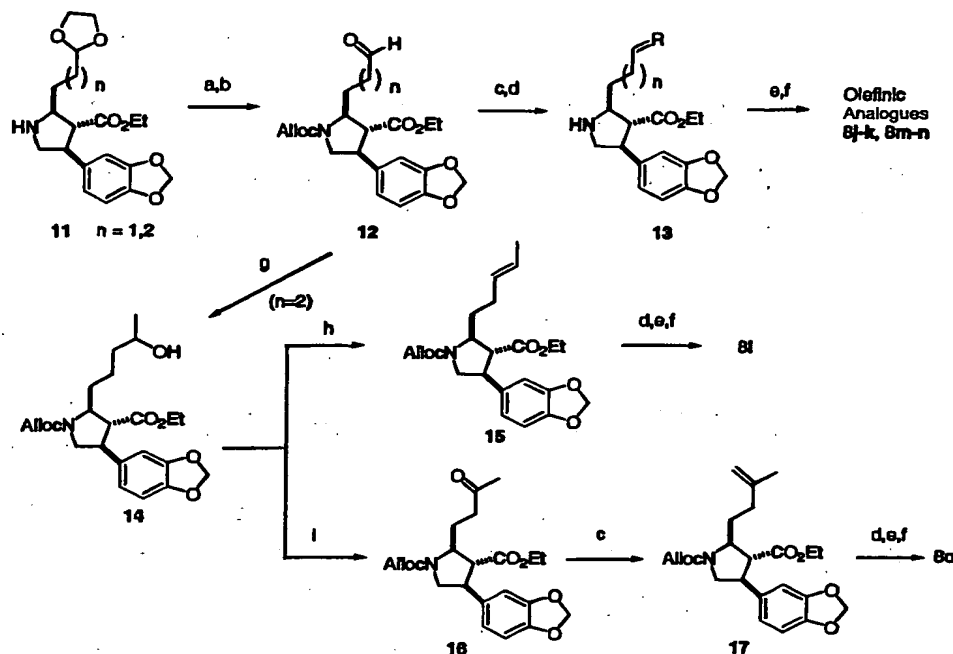
for ET<sub>A</sub> receptor.<sup>19</sup> The net result is a 13-fold improvement in ET<sub>A</sub> selectivity. The distinctive structure and selectivity feature of 3 served as a valuable lead for further exploration of this 2-alkylpyrrolidine-based, highly selective ET<sub>A</sub> receptor antagonists.

### Chemistry

Most of the new compounds in this report were synthesized by direct analogy to our earlier work on 1,<sup>18</sup> as shown in Scheme 2. Various  $\beta$ -ketoesters 4 were prepared according to the published procedures, through either reacting the imidazolidine of the carboxylic acid with magnesium monoethylmalonate followed by decarboxylation<sup>20</sup> (ca. 70% yield) or alkylating an acetoacetate dianion with an alkyl bromide<sup>21</sup> (20–70% yield). Condensation of nitromethane with piperonals provided the corresponding  $\beta$ -nitrostyrenes 5.<sup>22</sup> Potassium *tert*-butoxide catalyzed Michael addition of  $\beta$ -ketoesters 4 to nitrostyrenes 5 yielded two isomers of the adducts 6 in ca. 80% yield. Raney nickel mediated hydrogenation of the adduct 6 and reduction of the resultant cyclic imine with sodium cyanoborohydride provided a mixture of three diastereomeric pyrrolidines in which the desired

trans,trans isomer 7 predominated. The cis,cis isomer could be converted to trans,trans isomer by epimerization (DBU, CH<sub>3</sub>CN). Chromatographic separation on silica gel afforded the essentially pure trans,trans isomer of the amino ester 7 in ca. 40% yield from the Michael adduct 6. Alkylation of pyrrolidine 7 with *N,N*-dibutylbromoacetamide and subsequent hydrolysis of the ethyl ester furnished final compounds 8–10 in 80% yield for in vitro assays.

Compounds not suitable for the Raney nickel hydrogenation conditions (mainly olefin-containing substrates) were synthesized from dioxolanes 11 through a four-step sequence (Scheme 3). Protection of the pyrrolidine nitrogen as an allyl carbamate and hydrolysis of the acetal provided aldehyde 12. Wittig reaction of the resulting aldehyde 12 and removal of the urethane yielded the corresponding olefin substituted pyrrolidine 13, which was converted to the final antagonist as described in Scheme 2. Addition of methylmagnesium bromide to the aldehyde 12 and dehydration of the resulting alcohol 14 with Burgess reagent yielded the trans olefin 15. The alcohol 14 was also oxidized to ketone 16, which was converted to the terminal olefin 17 with an appropriate Wittig ylide.

Scheme 3<sup>a</sup>

<sup>a</sup> (a) Allyl chloroformate, py, DMAP, CH<sub>2</sub>Cl<sub>2</sub>; (b) 1 N aqueous HCl, THF, reflux; (c) Ph<sub>3</sub>P=R, THF; (d) Pd(Ph<sub>3</sub>P)<sub>4</sub>, Ph<sub>3</sub>P, pyrrolidine, CH<sub>2</sub>Cl<sub>2</sub>; (e) *n*-Bu<sub>2</sub>NCOCH<sub>2</sub>Br, *t*-Pr<sub>2</sub>NEt, CH<sub>3</sub>CN; (f) 6 N aqueous NaOH, EtOH; (g) MeMgBr, THF; (h) CH<sub>3</sub>O<sub>2</sub>CNSO<sub>2</sub>N(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>, CH<sub>3</sub>CN, heat; (i) PCC, MgSO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>.

Intermediates 15 and 17 were then converted to the desired antagonists via steps described previously in Scheme 2.

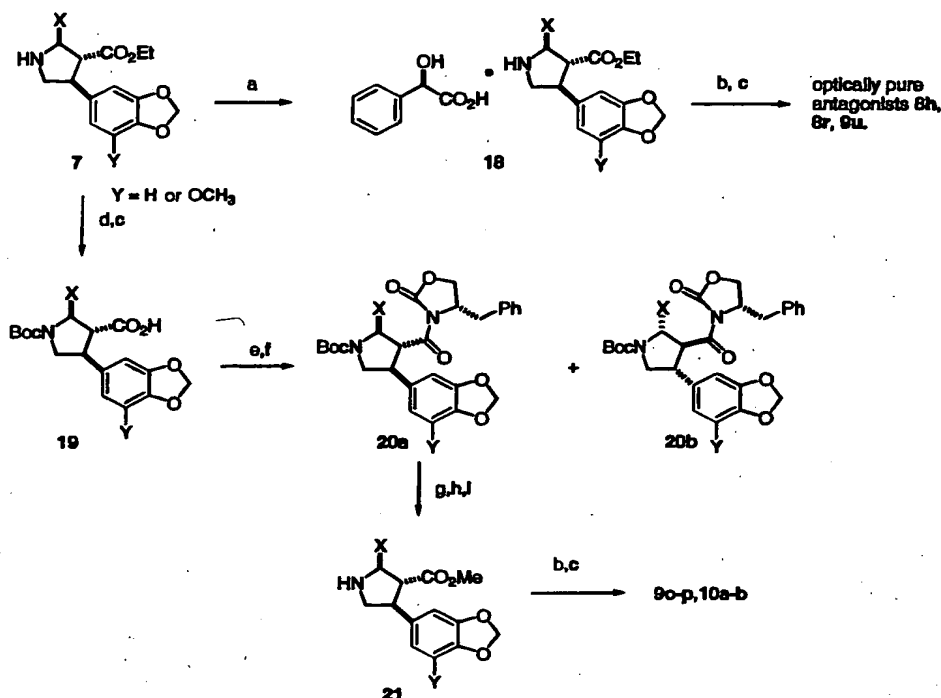
For detailed evaluation, a number of compounds have been prepared in optically pure form. The preparation of final products 8–10 as single enantiomers was generally accomplished through resolution of the pyrrolidine 7 prior to N-alkylation (Scheme 4). Certain racemic pyrrolidine esters 7 could be resolved via formation of chiral salts 18 with (*S*)-(+)-mandelic acid. Recrystallization produced material of >90% ee, as evaluated by chiral HPLC. The diastereomerically pure salt 18 was then converted to the optically pure final products as shown in Scheme 2. Alternatively, racemic pyrrolidine 7 could be converted to *N*-Boc carboxylic acid 19 in two steps. Acid 19 was then coupled with (*S*)-(-)-4-benzyl-2-oxazolidinone through a pentafluorophenylester to give diastereomers of 20a and 20b, which may be separated by silica flash chromatography. The desired acyl oxazolidinone 20a was then cleaved with sodium methoxide. Treatment of the resulting *N*-Boc methyl ester with trifluoroacetic acid and neutralizing the salt with aqueous base provided the optically pure pyrrolidine 21, which was converted to the final compounds.

### Structure–Activity Relationships

The initial screening for the compounds described in this study was a measurement of their ability to displace endothelin from its receptors. We employed a rodent ET<sub>A</sub> receptor derived from MMQ cells (*r*ET<sub>A</sub>); ET<sub>B</sub> receptor (*p*ET<sub>B</sub>) was derived from porcine cerebellar tissue. IC<sub>50</sub> data were recorded by measuring the displacement of [<sup>125</sup>I]ET-1 from the ET<sub>A</sub> receptor or [<sup>125</sup>I]-

ET-3 from the ET<sub>B</sub> receptor. Confirmatory binding studies were performed in a similar fashion, using human ET<sub>A</sub> and ET<sub>B</sub> receptor (*h*ET<sub>A</sub>, *h*ET<sub>B</sub>) permanently expressed in CHO cells.

The decreased ET<sub>B</sub> affinity of *n*-pentyl compound 3 suggested that the alkyl group might be finding a new hydrophobic binding site only present on the ET<sub>A</sub> receptor or the increased hydrophobicity of 3 was less tolerated at the ET<sub>B</sub> receptor than at the ET<sub>A</sub> receptor. In a separate study, the length of the linear alkyl groups was investigated.<sup>19</sup> The optimal chain length appears to be five atoms. Further shortening (propyl and butyl) or lengthening (hexyl and heptyl) of the alkyl chain was detrimental to both the ET<sub>A</sub> binding affinity and the ET<sub>B</sub>/ET<sub>A</sub> activity ratio. Only *n*-pentyl compound 3 provided reasonable affinity for ET<sub>A</sub> receptor and the largest separation of ET<sub>A</sub> and ET<sub>B</sub> activity. With the optimal chain length established, the substitution pattern of the aliphatic chain was then explored (Table 1). Only the smallest alkyl substituent, a methyl group, was tolerated at position 4 and 2 of the pentyl chain (8a and 8c), but not at position 3 or 1 (8b and 8d). For the larger substituents, 3-ethylpentyl group (8e) was 4 times less active and selective than 2-propylpentyl group (8f), and neither improved upon the parent *n*-pentyl compound 3. Fluorination at the terminal position of the pentyl chain has a slight negative effect on the affinity and selectivity of the antagonist 8g for the ET<sub>A</sub> receptor. Since a methyl group was tolerated at the 2-position of the alkyl chain, a *gem*-dimethyl group at the position 2 was introduced to eliminate the extra stereocenter. This modification (8h) slightly boosted the affinity for ET<sub>A</sub> receptor and decreased the ET<sub>B</sub> receptor

Scheme 4<sup>a</sup>

<sup>a</sup> (a) (S)-(+)-mandelic acid, Et<sub>2</sub>O-hexanes; (b) *n*-Bu<sub>2</sub>NCOCH<sub>2</sub>Br, *i*-Pr<sub>2</sub>NEt, CH<sub>3</sub>CN; (c) 6 N aqueous NaOH, EtOH; (d) Boc<sub>2</sub>O, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>; (e) pentafluorophenol, EDCI, DMF; (f) lithium salt of (S)-(-)-4-benzyl-2-oxazolidinone, THF, then silica gel chromatography; (g) NaOMe, MeOH; (h) TFA (neat); (i) aqueous NaHCO<sub>3</sub>, Et<sub>2</sub>O extraction.

affinity, thus tripling the receptor selectivity observed with antagonist 3.

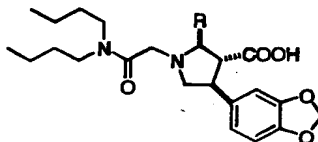
Double bonds were then introduced to the different positions of the pentyl chain to increase the rigidity of the aliphatic chain and to better position the alkyl group in the binding site (Table 1). Generally, double bonds between the position 3 and 4 were well tolerated. Compound **8i**, which includes a 4-methyl-3-pentenyl group, was slightly more potent and selective than *n*-pentyl analogue **3**. One carbon extension of the chain (**8j**) increased the binding affinity, but not the receptor selectivity. A more rigid derivative of **8i**, cyclopropylidenepropyl group (**8k**), exhibited a subnanomolar affinity for ET<sub>A</sub>, but without much improvement in selectivity. For the unsymmetric olefins, trans geometry seemed to have positive effects on increasing the affinity for ET<sub>A</sub> receptor, exemplified by (*E*)-3-pentenyl replacement **8l**. Unfortunately, compromised by the slightly increased ET<sub>B</sub> affinity, the subnanomolar ET<sub>A</sub> affinity of **8l** only doubled the receptor selectivity over lead compound **3**. On the other hand, transition from trans to cis geometry (**8m**) resulted in over 6-fold loss of ET<sub>A</sub> affinity and 3-fold loss of selectivity. Terminal olefins, both monosubstituted **8n** and disubstituted **8o**, have negative effects on the affinity and selectivity comparing to the aliphatic chain. Methyl substitution at the 3-position of trans olefin **8p** was tolerated. Compound **8q**, *gem*-dimethyl substituted **8i** at position 2, seemed to increase the selectivity for ET<sub>A</sub> receptor slightly. The optimal antagonist was realized through combining the two structural features of **8h** and **8l**, 2,2-dimethyl substitution and 3,4-trans olefin, to give **8r**, the first

antagonist with subnanomolar affinity and over 100000-fold selectivity for the ET<sub>A</sub> receptor versus the ET<sub>B</sub> receptor.

Because of the incompatibility of Raney nickel hydrogenation conditions with most of the olefinic compounds, 2-dioxolylalkyl substituted pyrrolidines **11** were prepared as the common intermediates for the olefin analogues (see Scheme 3). Surprisingly, alkylation of pyrrolidine **11** (*n* = 1) with the side chain of **1** and subsequent hydrolysis of the ester furnished potent (IC<sub>50</sub> = 2.3 nM) and highly selective (> 43500×) antagonist **9a** (Table 2). The structural difference between dioxylethyl and *n*-pentyl group intrigued us most, suggesting that we might have reached an alternative, hydrophilic binding site only present on the ET<sub>A</sub> receptor. The structure-activity relationship of this serendipitously discovered lead was then explored.

Initial derivatization of the 1,3-dioxolane ring offered disappointing results. Methyl substituents on the dioxolane ring (**9b**) were not tolerated. The 1,3-dioxane **9c** was less potent and selective than the 1,3-dioxolane **9a**. Compared to propylene linker (**9d**), ethylene appeared to be the optimal link between the heterocycles. Thus this chain length was kept constant for the ensuing analogues. Interestingly, transition from 1,3-dioxolane to 1,3-dithioxolane ring structure (**9e**) resulted in a 2-fold loss of the potency and over 6-fold decrease in selectivity for the ET<sub>A</sub> receptor, which strongly implicates hydrogen bonding as a factor contributing to the high selectivity. In contrast to the case of pentenyl compound **8r**, the introduction of a *gem*-dimethyl group at the α-carbon of the dioxolane (**9f**) only marginally

Table 1. SAR of 2-Acyclic Analogues: Radioligand Binding



compd	R	IC <sub>50</sub> (nM) <sup>a</sup>		B/A ratio <sup>b</sup>	formula
		rET <sub>A</sub> binding mean (range)	pET <sub>B</sub> binding mean (range)		
3	C <sub>4</sub> H <sub>9</sub> CH <sub>2</sub>	2.5 (3.3–1.8)	47000 (85000–26000)	18800	C <sub>27</sub> H <sub>42</sub> N <sub>2</sub> O <sub>5</sub> ·0.5H <sub>2</sub> O
8a	(CH <sub>3</sub> ) <sub>2</sub> CHC <sub>2</sub> H <sub>4</sub> CH <sub>2</sub>	2.5 (5.5–1.1)	35800 (38500–33000)	14300	C <sub>28</sub> H <sub>44</sub> N <sub>2</sub> O <sub>5</sub> ·0.65TFA
8b	C <sub>2</sub> H <sub>5</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	16 (31–8.1)	80000 (80000)	5000	C <sub>28</sub> H <sub>44</sub> N <sub>2</sub> O <sub>5</sub> ·0.60TFA·0.25Et <sub>2</sub> O
8c	C <sub>3</sub> H <sub>7</sub> CH(CH <sub>3</sub> )CH <sub>2</sub>	3.0 (3.3–2.8)	71000 (73300–68500)	23670	C <sub>28</sub> H <sub>44</sub> N <sub>2</sub> O <sub>5</sub> ·1.0TFA·0.5H <sub>2</sub> O
8d	C <sub>4</sub> H <sub>9</sub> C(CH <sub>3</sub> ) <sub>2</sub>	1300 (1800–950)	36000 (45800–28900)	28	C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>5</sub> ·1.60HCl
8e	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> CHCH <sub>2</sub> CH <sub>2</sub>	22 (88–9.9)	72000 (83950–55270)	3270	C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>5</sub> ·0.3TFA
8f	(C <sub>3</sub> H <sub>7</sub> ) <sub>2</sub> CHCH <sub>2</sub>	6.0 (28–2.3)	95000 (95000)	15800	C <sub>30</sub> H <sub>48</sub> N <sub>2</sub> O <sub>5</sub> ·0.35TFA
8g	F <sub>3</sub> CC <sub>3</sub> H <sub>7</sub> CH <sub>2</sub>	4.0 (4.2–3.8)	34000 (48300–24400)	8500	C <sub>27</sub> H <sub>39</sub> N <sub>2</sub> O <sub>5</sub> F <sub>3</sub> ·1.05TFA
8h	C <sub>3</sub> H <sub>7</sub> C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub>	1.3 (1.3–1.2)	62000 (72000–54000)	47690	C <sub>28</sub> H <sub>46</sub> N <sub>2</sub> O <sub>5</sub> ·1.05TFA
8i	(CH <sub>3</sub> ) <sub>2</sub> C=CHCH <sub>2</sub> CH <sub>2</sub>	1.3 (1.6–1.2)	33200 (38600–28500)	25500	C <sub>28</sub> H <sub>42</sub> N <sub>2</sub> O <sub>5</sub> ·0.05H <sub>2</sub> O·1.15TFA
8j	(C <sub>2</sub> H <sub>5</sub> )(CH <sub>3</sub> )C=CHCH <sub>2</sub> CH <sub>2</sub>	0.86 (1.5–0.4)	7000 (15800–3900)	8100	C <sub>29</sub> H <sub>44</sub> N <sub>2</sub> O <sub>5</sub> ·3.80TFA
8k	(CH <sub>2</sub> ) <sub>2</sub> C=CHCH <sub>2</sub> CH <sub>2</sub>	0.64 (0.83–0.49)	10000 (12500–8070)	15600	C <sub>28</sub> H <sub>40</sub> N <sub>2</sub> O <sub>5</sub> ·1.80TFA
8l	(E)-CH <sub>3</sub> CH=CHCH <sub>2</sub> CH <sub>2</sub>	0.78 (0.97–0.70)	26200 (52300–19000)	33600	C <sub>27</sub> H <sub>40</sub> N <sub>2</sub> O <sub>5</sub> ·0.85TFA
8m	(Z)-CH <sub>3</sub> CH=CHCH <sub>2</sub> CH <sub>2</sub>	4.8 (10.9–2.3)	67000 (79900–56700)	14000	C <sub>27</sub> H <sub>40</sub> N <sub>2</sub> O <sub>5</sub> ·1.30TFA
8n	CH <sub>2</sub> =CC <sub>2</sub> H <sub>4</sub> CH <sub>2</sub>	9.9 (39–4.3)	60000 (73200–51600)	6060	C <sub>27</sub> H <sub>40</sub> N <sub>2</sub> O <sub>5</sub> ·1.10TFA·1.05AcOH
8o	CH <sub>2</sub> =C(CH <sub>3</sub> )C <sub>2</sub> H <sub>4</sub> CH <sub>2</sub>	5.2 (5.7–4.8)	36000 (36000)	6900	C <sub>28</sub> H <sub>42</sub> N <sub>2</sub> O <sub>5</sub> ·1.30TFA
8p	(E)-CH <sub>3</sub> CH=C(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	3.3 (6.8–1.7)	48000 (48000)	14500	C <sub>28</sub> H <sub>42</sub> N <sub>2</sub> O <sub>5</sub> ·0.70TFA
8q	(CH <sub>3</sub> ) <sub>2</sub> C=CHC(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub>	2.7 (3.2–2.3)	64000 (64000)	23700	C <sub>30</sub> H <sub>46</sub> N <sub>2</sub> O <sub>5</sub> ·1.05TFA
8r (2S,3R,4S)	(E)-CH <sub>3</sub> CH=CHC(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub>	0.78 (1.1–0.57)	>100000	>128000	C <sub>29</sub> H <sub>44</sub> N <sub>2</sub> O <sub>5</sub> ·0.70TFA

<sup>a</sup> IC<sub>50</sub> calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10<sup>-10</sup> to 10<sup>-5</sup> unless otherwise noted. <sup>b</sup> Expressed as IC<sub>50</sub>(ET<sub>B</sub>)/IC<sub>50</sub>(ET<sub>A</sub>).

augmented the affinity and the selectivity. This result is consistent with our hypothesis that the hydrophobic and hydrophilic substituents bind to different sites of the ET<sub>A</sub> receptor.

A pharmacokinetic study of the compound 9a (in rats) indicated some hydrolysis of the dioxolane to the aldehyde in vivo. To eliminate the acid labile dioxolane moiety, heterocycles mimicking the dioxolane ring structure were sought. By replacing one of the oxygens of the dioxolane ring with carbon, furan and pyran derivatives were synthesized. Among this group, the best compound in terms of affinity and specificity for the ET<sub>A</sub> receptor was the 2-tetrahydropyranyl compound 9g, with slightly improved ET<sub>A</sub> affinity and decreased receptor selectivity. Moving the oxygen to the position 4 of the ring resulted in antagonist 9h with much lower affinity for the ET<sub>A</sub> receptor. For the five-membered ring system, 2-furan 9i was equally active and selective as 3-furan 9j. The fully reduced 2-tetrahydrofuran 9k was 6 times less active than the tetrahydropyran 9g.

Dioxolane replacements oxazoles and pyrazole were also examined. Oxazole 9l showed a decreased ET<sub>B</sub> affinity while maintaining the ET<sub>A</sub> affinity, which led to an antagonist with over 41700-fold selectivity for the ET<sub>A</sub> receptor. Despite an overall increase in potency, dimethyl substituted oxazole 9m showed a greater increase of ET<sub>B</sub> affinity than ET<sub>A</sub> affinity. Compound 9n, a N-methylated pyrrole, did not offer any improvement over the 1,3-dioxolane. A more dramatic effect was seen in 2-pyridyl compound 9o, which has subnanomolar affinity and almost 40000-fold selectivity for the ET<sub>A</sub> receptor over the ET<sub>B</sub> receptor.

On the basis of the active pyridine compound 9o, a strategy of heteroatom frame shift was conceived. The ethylene linker was moved to the nitrogen of the heterocycle and the original connecting carbon center was changed to nitrogen or a carbonyl group. Compound 9p, a pyrazole replacement, offered subnanomolar affinity and improved selectivity compared to 1,3-dioxolane 9a. Succinimide 9q was equally potent and

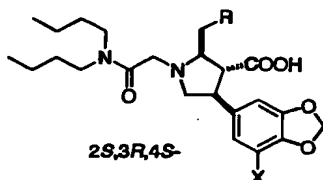
CCCCN(CCCC)C(=O)CN1C[C@H](C(=O)O)[C@@H](C1Cc2ccc3c(c2)OCO3)C1CC1R

<sup>a</sup> IC<sub>50</sub> calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10<sup>-10</sup> to 10<sup>-5</sup> unless otherwise noted. <sup>b</sup> Expressed as IC<sub>50</sub>(ET<sub>B</sub>)/IC<sub>50</sub>(ET<sub>A</sub>).

## Secondary Evaluations

After synthesizing a series of very potent and highly selective ET<sub>A</sub> antagonists with varying hydrophobicity and hydrophilicity, a small set of compounds were prepared in enantiomerically pure form to evaluate the impact of this polarity swing on the pharmacokinetic profiles of the compounds. These compounds were first evaluated in our standard screening assay, as well as in a second set of binding assays employing human ET<sub>A</sub> and ET<sub>B</sub> receptors expressed in CHO cells (Table 3). As we have observed in previous studies,<sup>18,23</sup> the *r*ET<sub>A</sub> and *p*ET<sub>B</sub> receptors used in the screening assays reliably predict the relative and absolute affinities observed

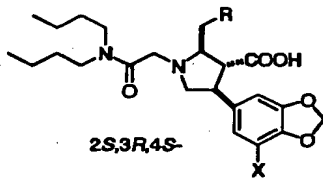
Table 3. Summary Data for Single Enantiomers



compd	R	X	binding IC <sub>50</sub> (nM) <sup>a,b</sup>		B/A ratio <sup>d</sup>	binding IC <sub>50</sub> (nM) <sup>a,c</sup>		B/A ratio <sup>d</sup>	formula
			rET <sub>A</sub> mean (range)	pET <sub>B</sub> mean (range)		hET <sub>A</sub> mean (range)	hET <sub>B</sub> mean (range)		
8h	C <sub>3</sub> H <sub>7</sub> C(CH <sub>3</sub> ) <sub>2</sub>	H	0.67 (0.96–0.45)	2900 (5900–1600)	4300	0.29 (0.37–0.23)	2800 (4300–1900)	9700	nd
8r	(E)-CH <sub>3</sub> CH=CHC(CH <sub>3</sub> ) <sub>2</sub>	H	0.78 (1.1–0.57)	>100000	>128000	1.0 (1.3–0.77)	92700 (92700)	92700	C <sub>29</sub> H <sub>41</sub> N <sub>2</sub> O <sub>5</sub> •0.70TFA
9o	(2-pyridyl)CH <sub>2</sub>	H	0.72 (1.4–0.35)	26000 (36000–15000)	36000	0.29 (0.68–0.12)	32000 (41000–25000)	110000	C <sub>29</sub> H <sub>39</sub> N <sub>3</sub> O <sub>5</sub> •2.25TFA•1.10H <sub>2</sub> O
9p	(1-pyrazolyl)CH <sub>2</sub>	H	0.68 (0.71–0.66)	26200 (33000–20600)	38500	1.06 (1.67–0.68)	42000 (83800–21100)	39600	C <sub>27</sub> H <sub>39</sub> N <sub>4</sub> O <sub>5</sub> •0.90TFA
9u	(2-oxopyrrolidinyl)CH <sub>2</sub>	H	0.22 (0.25–0.20)	24000 (27700–20600)	110000	0.17 (0.18–0.17)	18000 (23000–13800)	106000	C <sub>28</sub> H <sub>41</sub> N <sub>3</sub> O <sub>6</sub> •0.85TFA
10a	C <sub>3</sub> H <sub>7</sub> C(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	0.56 (0.86–0.33)	16700 (24900–9100)	29800	0.49 (0.61–0.41)	15400 (20000–12400)	31400	C <sub>30</sub> H <sub>43</sub> N <sub>2</sub> O <sub>6</sub> •0.7TFA
10b	(E)-CH <sub>3</sub> CH=CHC(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	0.41 (0.41–0.40)	45300 (81000–23000)	110000	0.29 (0.68–0.13)	39700 (73300–27000)	137000	C <sub>30</sub> H <sub>45</sub> N <sub>2</sub> O <sub>6</sub> •0.80TFA

<sup>a</sup> IC<sub>50</sub> calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10<sup>-10</sup> to 10<sup>-5</sup> unless otherwise noted. <sup>b</sup> Binding assays recorded as described in Experimental Section, using MMQ cells (rET<sub>A</sub>), porcine cerebellar tissue (pET<sub>B</sub>). <sup>c</sup> Binding assays recorded as described in Experimental Section, using clonal CHO cell lines (hET<sub>A</sub> and hET<sub>B</sub>). <sup>d</sup> Expressed as IC<sub>50</sub>(ET<sub>B</sub>)/IC<sub>50</sub>(ET<sub>A</sub>).

Table 4. Summary of Pharmacokinetic Profiles for Single Enantiomers



compd	R	X	pharmacokinetic profiles (rats) <sup>a</sup>				
			T <sub>1/2</sub> iv (h)	T <sub>1/2</sub> oral (h)	C <sub>max</sub> (μg/mL)	AUC (μg h/mL)	F (%)
8h	C <sub>3</sub> H <sub>7</sub> C(CH <sub>3</sub> ) <sub>2</sub>	H	2.45	3.85	1.48	7.06	76.8
8r	(E)-CH <sub>3</sub> CH=CHC(CH <sub>3</sub> ) <sub>2</sub>	H	1.6	3.58	1.49	2.62	32.2
9o (racemic)	(2-pyridyl)CH <sub>2</sub>	H	2.8	8.8	0.58	0.78	21.1
9u	(2-oxopyrrolidin-1-yl)CH <sub>2</sub>	H	1.4	nd	0.016	0.009	0.3
10a	C <sub>3</sub> H <sub>7</sub> C(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	1.6	2.5	1.69	3.27	47.7
10b	(E)-CH <sub>3</sub> CH=CHC(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	1.5	3.94	0.66	2.09	27.8

<sup>a</sup> T<sub>1/2</sub> iv, half-life after intravenous dosing. T<sub>1/2</sub> oral, C<sub>max</sub>, AUC, and F are half-life, maximum drug concentration, total drug exposure (area under the curve) and oral bioavailability after oral dosing in rats. Calculated from raw pharmacokinetic data as described in the Experimental Section.

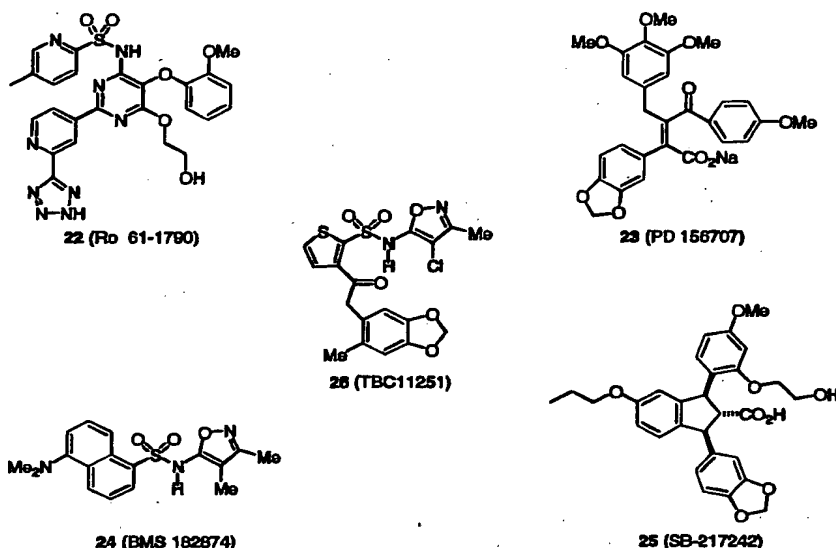
against human receptors, with some tendency for this class of compounds to exhibit a higher B/A ratio for the human genotypes. Most of the compounds in this group have subnanomolar potencies against hET<sub>A</sub> receptors; B/A ratios vary from 9700 to 137000.

Pharmacokinetic studies (in rats) of these enantiomers revealed that the vastly different pharmacokinetic profiles of these two series were associated with their distinctively different physical properties (Table 4). Oral bioavailability of 8h is very good (77%) but with a very slow absorption rate, which could be explained by the extremely high hydrophobicity of the compound, compared to 1. Compound 8r has much lower bioavailability and a much smaller AUC values. On the other hand, the very hydrophilic series of compounds, exemplified by 9o and 9u, exhibit very poor pharmacokinetic

profiles: low oral bioavailabilities and small C<sub>max</sub> and AUC values. Rapid elimination and a tendency toward oxidation might contribute to the poor showing of oral bioavailability of a number of these compounds.

A separate unpublished SAR study by our group has indicated that the placement of a methoxy group at the position 7 of the benzodioxolane ring was well tolerated for the ET<sub>A</sub> selective antagonists. To improve the absorption rate of compounds 8h and 8r, slightly more hydrophilicity was added to the alkyl series by introducing this modification. Initial biochemical assays indicated improved binding affinity and receptor selectivity for new analogs 10a (A-216546) and 10b over the parent compounds (Table 3). In fact, compound 10b is arguably the most ET<sub>A</sub> selective antagonist known to date against human receptors.

Chart 1



The results of pharmacokinetic profile change of these two compounds were mixed. The hydrophilicity modification improved the pharmacokinetic profiles of 10a, but not of 10b. The absorption rate of 10a increased with improved hydrophilicity, comparing to compound 8h. Compound 10a, is also well absorbed (oral bioavailability  $F = 48\%$ ), despite its relatively short iv half-life (1.6 h). In the phase I single dose human study, ABT-627 (**1**) exhibits a much longer elimination half-life (24 h) than that expected from the rat study (3.5 h). Both in vitro and in vivo metabolism studies have suggested that one of the major metabolic routes for **1** appears to be the glucuronidation of the carboxylic acid moiety. The in vivo studies with [<sup>14</sup>C]A-127722 indicates that the drug is recovered through enterohepatic recycling of the glucuronide of **1**, which explains its prolonged half-life in human. Apparently, this recycling process is much more profound in human than in rat. If the same trend holds, a longer half-life for 10a in human than in rat is expected. The ability of 10a to inhibit ET-1 stimulated phosphoinositide (PI) hydrolysis was also determined. Compound 10a effectively inhibits ET-1 evoked PI hydrolysis in rat MMQ cells with an IC<sub>50</sub> value of 0.59 nM, showing the compound indeed is a very potent functional antagonist of endothelin.

Since 1993, a number of non-peptide ET<sub>A</sub> selective antagonists have been reported (Chart 1), including Ro 61-1790<sup>24</sup> (**22**), PD 156707<sup>25</sup> (**23**), BMS 182874<sup>26</sup> (**24**), SB 217242<sup>27</sup> (**25**), and TBC11251<sup>28</sup> (**26**). Except for **24**, all of these compounds exhibit subnanomolar affinities for the ET<sub>A</sub> receptor (Table 5). The selectivity of these antagonists for human ET<sub>A</sub> receptor is about 1000-fold, with the exception of **25** and **26**. Compound **26** is the most selective agent (>10000-fold for ET<sub>A</sub> vs ET<sub>B</sub>) reported to date and is well-absorbed orally (60% in rats). By comparison, compound 10a appears to be as potent against the ET<sub>A</sub> receptor as any of the above antagonists; it also shows the least affinity for ET<sub>B</sub> receptor, which translates into the largest ET<sub>B</sub>/ET<sub>A</sub> ratio (over 30000).

Table 5. Comparison Data for Representative ET<sub>A</sub> Selective Antagonists

compd	K <sub>i</sub> (nM) <sup>a</sup>		B/A ratio <sup>b</sup>	iv half-life <sup>c</sup> (h)	F <sup>c</sup> (%)
	hET <sub>A</sub> binding	hET <sub>B</sub> binding			
10a	0.46	13000	28260	1.6	48
22	0.13	175	1346	0.8	N/A
23	0.17	139	818	1.0	41
24	48	>50000	>1040	N/A	N/A
25	1.1	111	100	3.3	66
26	0.43	>4300	>10000	6.7	60

<sup>a</sup> Human receptor data acquired in a variety of systems, collected from a variety of sources. <sup>b</sup> Expressed as K<sub>i</sub>(ET<sub>B</sub>)/K<sub>i</sub>(ET<sub>A</sub>). <sup>c</sup> T<sub>1/2</sub> and oral bioavailability (F) data, measured in rats, collected from a variety of sources.

## Conclusions

Two novel series of pyrrolidine-3-carboxylic acid based highly ET<sub>A</sub> selective antagonists have been identified through modification of the *p*-anisyl group of the ET<sub>A</sub>-selective endothelin antagonist **1**. In the first series, structure-activity studies revealed that the *p*-anisyl group of **1** could be replaced with hydrophobic 2,2-dimethylpentyl (**8h**) and 2,2-dimethylpentenyl (**8r**) groups. The resultant analogues retain the high ET<sub>A</sub> affinity of **1**, but exhibit substantially reduced ET<sub>B</sub> activity. In particular, the combination of a 2,2-dimethylpentenyl group, *N,N*-dibutylacetamide side chain, and a 7-methoxy-1,3-benzodioxol-5-yl group provides antagonist 10b with subnanomolar affinity for the ET<sub>A</sub> receptor and over 100000-fold selectivity. A number of these compounds also exhibit oral bioavailabilities (in rats) in the 28–77% range and have good plasma half-lives. Of these, compound 10a, exhibits the best combination of biochemical and pharmacological properties, and has been identified as a potential backup to **1**.

For the alkyl heterocycle series, it was found that the size of the heterocycles and positioning of the hydrogen bonding acceptors are important in optimizing this series of highly selective antagonists. In particular, 2-pyridylethyl (**9o**) and 2-(2-oxopyrrolidinyl)ethyl (**9u**) groups have the best combination of potency and



selectivity. Unfortunately, poor pharmacokinetic profiles are generally exhibited by these very hydrophilic compounds, which prevent them from being orally dosed.

The highly selective receptor-binding profile of these potent and orally bioavailable compounds further improved the  $ET_A$  selectivity observed with 1. It remains to be seen whether reduced  $ET_B$  antagonism will prove to be advantageous in treating diseases in which endothelin-1 plays a pathogenic role.

## Experimental Section

**General.** Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions were performed under nitrogen atmosphere unless specifically noted. Flash chromatography was performed using silica gel (230–400 mesh) from E. M. Science. Proton NMR spectra were recorded on a General Electric QE300 instrument with  $Me_4Si$  as an internal standard and are reported as shift (multiplicity, coupling constants, proton counts). Mass spectral analyses were accomplished using different techniques, including direct chemical ionization (DCI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), and fast atom bombardment (FAB), as specified for individual compounds. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ, and are consistent with theoretical values to within 0.4% unless indicated.

**Ethyl 2-(*n*-Hexanoyl)-3-(nitromethyl)-1,3-benzodioxol-5-propionate (6,  $X = C_6H_5CH_2$ ,  $Y = H$ ).** To a solution of ethyl 3-oxo-6-octanoate (5.0 g, 26.9 mmol) in 100 mL of THF was added 3,4-methylenedioxy- $\beta$ -nitrostyrene (5.1 g, 26.4 mmol), followed by the addition of potassium *tert*-butoxide (30 mg, 0.27 mmol). The mixture was stirred at room temperature for 2 h, during which the solid dissolved and the solution became reddish. After 2 h, TLC (ethyl acetate–hexanes, 1:5) indicated complete consumption of the ketoester. The solution was then concentrated in vacuo, and the residue was flash chromatographed on silica gel eluting with 20% ethyl acetate in hexane to produce 8.7 g (23.0 mmol, 85%) of the title compound 6 as a mixture of diastereomers in a 1:1 ratio.

***trans,trans*-Ethyl 2-(*n*-Pentyl)-4-(1,3-benzodioxol-5-yl)-pyrrolidine-3-carboxylate (7,  $X = C_6H_5CH_2$ ,  $Y = H$ ).** The nitro ketone 6 (8.7 g, 23.0 mmol) in 150 mL of ethyl acetate was hydrogenated under 4 atm of hydrogen pressure using a Raney nickel 2800 catalyst (8.7 g). The Raney nickel was washed sequentially with methanol, THF, and EtOAc before use. The catalyst was then removed by filtration, and the solution was concentrated under reduced pressure. The resulting crude imine (7.7 g, 23.3 mmol) was dissolved in 25 mL of tetrahydrofuran and 50 mL of ethanol. Sodium cyanoborohydride (1.6 g, 25.5 mmol) and 2 mg of bromocresol green were added. To this blue solution was added dropwise a solution of 1:2 concentrated HCl in ethanol at such a rate that the color was kept at light yellow-green. After the yellow color persisted without additional HCl, the solution was stirred for an additional 40 min. The solution was then concentrated in vacuo and partitioned between chloroform and an aqueous saturated sodium bicarbonate solution. The organic phase was separated, dried over sodium sulfate, and concentrated under reduced pressure. The residue was dissolved in 35 mL of acetonitrile and DBU (4.3 g, 28.2 mmol) was added. The solution was refluxed overnight. TLC (EtOAc) showed no more *cis,cis* isomer. The solvent was removed in vacuo and the crude product was flash chromatographed on silica gel eluting with 75:25 ethyl acetate–hexane to give 3.5 g of pure *trans,trans* compound 7 (10.5 mmol, 46% from 6) as a light yellow oil.

**Resolution of Compounds.** Method A. *trans,trans*-Ethyl [2*S*,3*R*,4*S*]-2-(2,2-Dimethylpentyl)-4-(1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylate (18,  $X = C_6H_5CH_2$ ,  $Y = H$ ). The amino ester 7 (6.8 g, 18.8 mmol) was dissolved

in 100 mL of ether; a solution of 1.6 g (10.5 mmol) of (*S*)-(+)-mandelic acid in 60 mL of ether was added, the total volume was made up to ~200 mL, and the solution was seeded. The mixture was stirred slowly overnight. The resultant crystals were collected by filtration and recrystallized from ether/EtOAc to give 1.8 g (3.5 mmol, 37%) of a white solid. This material was partitioned between sodium bicarbonate and ether; the ether layer was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give the enantiomerically pure product (>98% ee), as indicated by chiral HPLC analysis, using a Regis Whelk-O column.

**Method B. *trans,trans*-Methyl [2*S*,3*R*,4*S*]-2-(2,2-Dimethylpentyl)-4-(7-methoxy-1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylate (21,  $X = C_6H_5C(CH_3)_2CH_2$ ,  $Y = OCH_3$ ).** To a solution of crude amino ester 7 (2.0 g, 5.1 mmol) in 40 mL of dichloromethane with 4 mL (28.7 mmol) of triethylamine was added 2.0 g (9.2 mmol) of di-*tert*-butyl dicarbonate, and the mixture was stirred at ambient temperature for 5 h. Solvents were then removed in vacuo, and the residue was taken up in 60 mL of ethanol. Aqueous sodium hydroxide (10 mL of 2.5 N solution, 25 mmol) was added, and the resultant solution was stirred overnight. Solvents were removed in vacuo; the residue was taken up in water and extracted with ether. The aqueous phase was acidified with aqueous 1 N phosphoric acid and extracted with EtOAc. The organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated to give 1.0 g of a colorless oil. A sample of this material (0.734 g, 1.58 mmol) was combined with 0.35 g (1.9 mmol) of pentafluorophenol and 0.364 g (1.9 mmol) of EDAC in 5 mL of DMF. The resultant solution was stirred at ambient temperature for 1 h, then was poured onto 50 mL of 0.6 M sodium bicarbonate solution and extracted (3 × 15 mL) with ether. The combined ether extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give a foam, which was dissolved in 5 mL of THF and cooled to 0 °C. Simultaneously, 0.418 g (2.37 mmol) of (*S*)-(-)-4-benzyl-2-oxazolidinone was combined with ~0.1 mg of pyreneacetic acid in 5 mL of THF and cooled to 0 °C. *N*-Butyllithium (1.6 M in hexanes) was added to a red endpoint (persists ~10 s), and the solution was stirred for 10 min. The solution was transferred into the THF solution of the pentafluorophenyl ester, and the resultant solution was stirred at 0 °C for 40 min. Solvents were removed in vacuo; the residue was taken up in sodium bicarbonate and extracted with ether (3 × 10 mL). The combined ether extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude mixture of diastereomeric products was separated by flash chromatography on silica gel, eluting with a gradient from 4:1 → 3:1 → 2:1 hexanes/EtOAc, giving 423 mg (0.68 mmol) of the faster moving and 389 mg (0.63 mmol) of the slower moving diastereomer, respectively. The faster moving diastereomer was dissolved in 2 mL of a 2.0 M solution of sodium methoxide (4.0 mmol) in methanol (freshly prepared, containing 5% methyl formate by volume) and stirred at ambient temperature for 16 h. Solvents were removed in vacuo, and the residue was partitioned between ether and aqueous 1 N sodium hydroxide. The ether layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with 4:1 hexanes/EtOAc. The resultant material was dissolved in 5 mL of TFA and stirred at ambient temperature for 1 h. Solvents were removed in vacuo; the residue was suspended in sodium bicarbonate solution and extracted with EtOAc. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give 98 mg (0.25 mmol, 33% from the *N*-Boc acid) of the resolved amino ester.

***N,N*-Dibutylbromoacetamide.** The bromoacetamide employed in this study was prepared using the method of Weaver, as described in our earlier work.

***trans,trans*-2-(*n*-Pentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic acid (3).** The pyrrolidine 7 (48 mg, 0.14 mmol) was combined with 35 mg (0.14 mmol) of the *N,N*-dibutylbromoac-

etamide in 3 mL of acetonitrile; 0.5 mL (2.9 mmol) of Hünig's base was added, and the solution was allowed to stir overnight at ambient temperature. Solvents were removed in vacuo; the residue was partitioned between EtOAc and aqueous 1 N phosphoric acid. The organic layer was washed with sodium bicarbonate solution and brine, then dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 2:1 hexanes/EtOAc. The product was dissolved in 4 mL of ethanol; 1 mL of 2.5 N aqueous sodium hydroxide (2.5 mmol) was added, and the resultant solution was stirred overnight at ambient temperature. Solvents were removed in vacuo; the residue was taken up in water and extracted with ether. The aqueous phase was acidified to pH 5 with aqueous 1 N phosphoric acid and extracted with EtOAc. The organic extracts were washed with brine, dried over sodium sulfate, and filtered. The solvents were removed in vacuo to give 56 mg (0.12 mmol, 86%) of the title compound as white foam:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.87 (t,  $J$  = 7.5 Hz, 3H), 0.89 (t,  $J$  = 7.5 Hz, 3H), 0.97 (t,  $J$  = 7.5 Hz, 3H), 1.21–1.42 (brm, 10H), 1.43–1.78 (brm, 6H), 2.76 (t,  $J$  = 7.0 Hz, 1H), 3.02–3.30 (brm, 6H), 3.40–3.60 (m, 3H), 3.73 (d,  $J$  = 14.0 Hz, 1H), 5.98 (AB, 2H), 6.70 (d,  $J$  = 8.1 Hz, 1H), 6.77 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.89 (d,  $J$  = 1.8 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  475. Anal. Calcd for  $\text{C}_{27}\text{H}_{44}\text{N}_2\text{O}_5 \cdot 0.5\text{H}_2\text{O}$ : C, 67.05; H, 8.96; N, 5.79. Found: C, 67.30; H, 8.77; N, 5.68.

The following compounds were prepared using the procedures described above for compound 3.

**trans,trans-2-(4-Methylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8a):** an amorphous solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.83 (d,  $J$  = 6.9 Hz, 3H), 0.84 (d,  $J$  = 6.9 Hz, 3H), 0.91 (t,  $J$  = 7.5 Hz, 3H), 0.96 (t,  $J$  = 7.5 Hz, 3H), 1.13–1.90 (m, 15H), 3.00–4.20 (m, 11H), 5.93 (s, 2H), 6.74 (d,  $J$  = 8.1 Hz, 1H), 6.78 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.87 (d,  $J$  = 1.8 Hz, 1H); MS (APCI) ( $M + \text{H}^+$ ) at  $m/z$  489. Anal. Calcd for  $\text{C}_{28}\text{H}_{44}\text{N}_2\text{O}_5 \cdot 0.65\text{TFA}$ : C, 62.53; H, 8.00; N, 4.98. Found: C, 62.50; H, 8.02; N, 4.96.

**trans,trans-2-(3-Methylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8b):** an amorphous solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.83 (d,  $J$  = 6.9 Hz, 3H), 0.84 (d,  $J$  = 6.9 Hz, 3H), 0.91 (t,  $J$  = 7.5 Hz, 3H), 0.96 (t,  $J$  = 7.5 Hz, 3H), 1.05–1.22 (m, 2H), 1.22–1.41 (m, 7H), 1.43–1.68 (m, 5H), 1.89 (m, 1H), 2.94 (t,  $J$  = 6.0 Hz, 1H), 3.15–3.27 (m, 3H), 3.29–3.60 (m, 5H), 3.72 (brd,  $J$  = 6.0 Hz, 1H), 3.92 (brd,  $J$  = 13.5 Hz, 1H), 5.93 (dd,  $J$  = 2.0, 4.0 Hz, 2H), 6.73 (d,  $J$  = 8.1 Hz, 1H), 6.78 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.88 (d,  $J$  = 1.8 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  489. Anal. Calcd for  $\text{C}_{28}\text{H}_{44}\text{N}_2\text{O}_5 \cdot 0.60\text{TFA} \cdot 0.25\text{Et}_3\text{O}$ : C, 66.08; H, 9.02; N, 4.99. Found: C, 65.93; H, 8.81; N, 4.84.

**trans,trans-2-(2-Methylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)aminocarbonylmethyl]pyrrolidine-3-carboxylic Acid (8c):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.8–1.0 (m, 12H), 1.20–1.40 (m, 7H), 1.45–1.60 (m, 6H), 1.60–1.74 (m, 1H), 1.80–2.0 (m, 1H), 3.10–3.40 (m, 5H), 3.67–3.78 (m, 1H), 3.80–3.91 (m, 1H), 4.0–4.20 (m, 2H), 4.30–4.50 (m, 2H), 5.93 (d,  $J$  = 1.5 Hz, 2H), 6.73 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.79 (ddd,  $J$  = 1.8, 1.8, 8.1 Hz, 1H), 6.86 (dd,  $J$  = 1.8, 3.9 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  489. Anal. Calcd for  $\text{C}_{28}\text{H}_{44}\text{N}_2\text{O}_5 \cdot 1.0\text{TFA} \cdot 0.5\text{H}_2\text{O}$ : C, 58.91; H, 7.58; N, 4.58. Found: C, 58.91; H, 7.58; N, 4.45.

**trans,trans-2-(1,1-Dimethylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8d):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.93 (t,  $J$  = 7.5 Hz, 3H), 0.94 (t,  $J$  = 7.5 Hz, 3H), 0.95 (t,  $J$  = 7.5 Hz, 3H), 1.06 (s, 3H), 1.12 (s, 3H), 1.20–1.42 (m, 10H), 1.44–1.68 (m, 4H), 3.16–3.60 (m, 6H), 3.91 (brdd,  $J$  = 6.0, 11.4 Hz, 1H), 4.08 (d,  $J$  = 16.8 Hz, 1H), 4.27 (m, 1H), 4.33 (d,  $J$  = 9.0 Hz, 1H), 5.87 (brd,  $J$  = 18.0 Hz, 1H), 5.94 (s, 2H), 6.70 (d,  $J$  = 8.1 Hz, 1H), 6.82 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.85 (d,  $J$  = 1.8 Hz, 1H); MS (APCI/ $\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  503. Anal. Calcd for  $\text{C}_{29}\text{H}_{46}\text{N}_2\text{O}_5 \cdot 1.60\text{HCl}$ : C, 62.09; H, 8.55; N, 4.99. Found: C, 62.09; H, 8.21; N, 4.65.

**trans,trans-2-(3-Ethylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8e):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.81 (t,  $J$  = 7.5 Hz, 6H), 0.90 (t,  $J$  = 7.5 Hz, 3H), 0.96 (t,  $J$  = 7.5 Hz, 3H), 1.10–1.42 (m, 12H), 1.43–1.62 (m, 4H), 1.68–1.72 (brm, 1H), 2.91 (brt,  $J$  = 7.5 Hz, 1H), 3.14–3.28 (m, 2H), 3.28–3.52 (m, 6H), 3.70 (brd,  $J$  = 6.6 Hz, 1H), 3.92 (brd,  $J$  = 16.5 Hz, 1H), 5.92 (dd,  $J$  = 2.0, 4.0 Hz, 2H), 6.71 (d,  $J$  = 8.1 Hz, 1H), 6.79 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.88 (d,  $J$  = 1.8 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  503. Anal. Calcd for  $\text{C}_{29}\text{H}_{46}\text{N}_2\text{O}_5 \cdot 0.3\text{TFA}$ : C, 66.22; H, 8.69; N, 5.22. Found: C, 66.40; H, 9.08; N, 5.10.

**trans,trans-2-(2-Propylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8f):** an amorphous solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.85 (m, 6H), 0.92 (t,  $J$  = 7.5 Hz, 3H), 0.97 (t,  $J$  = 7.5 Hz, 3H), 1.12–1.40 (m, 13H), 1.42–1.68 (m, 6H), 2.90 (m, 1H), 3.14–3.30 (m, 3H), 3.33 (m, 5H), 3.72 (brm, 1H), 3.90 (brm, 1H), 5.93 (dd,  $J$  = 2.0, 4.0 Hz, 2H), 6.73 (d,  $J$  = 8.1 Hz, 1H), 6.78 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.88 (d,  $J$  = 1.8 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  517. Anal. Calcd for  $\text{C}_{30}\text{H}_{48}\text{N}_2\text{O}_5 \cdot 0.35\text{TFA}$ : C, 66.24; H, 8.76; N, 5.03. Found: C, 66.26; H, 8.82; N, 4.98.

**trans,trans-2-(5,5,5-Trifluoropentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8g):** an amorphous solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.92 (t,  $J$  = 7.5 Hz, 3H), 0.95 (t,  $J$  = 7.5 Hz, 3H), 1.31 (septet,  $J$  = 7.5 Hz, 4H), 1.41–1.66 (m, 8H), 1.76–2.15 (m, 4H), 3.10–3.25 (m, 3H), 3.30 (dd,  $J$  = 6.0, 12.6 Hz, 1H), 3.40 (dd,  $J$  = 6.0, 12.6 Hz, 1H), 3.67 (t,  $J$  = 10.8 Hz, 1H), 3.77 (t,  $J$  = 10.8 Hz, 1H), 3.99 (dd,  $J$  = 9.9, 19.2 Hz, 1H), 4.03 (d,  $J$  = 16.5 Hz, 1H), 4.23–4.33 (m, 1H), 4.35 (d,  $J$  = 16.5 Hz, 1H), 5.94 (s, 2H), 6.73 (d,  $J$  = 8.1 Hz, 1H), 6.79 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.82 (d,  $J$  = 1.8 Hz, 1H); MS (ESI) ( $M + \text{H}^+$ ) at  $m/z$  529. Anal. Calcd for  $\text{C}_{27}\text{H}_{39}\text{N}_2\text{O}_5\text{F}_3 \cdot 1.05\text{TFA}$ : C, 53.91; H, 6.23; N, 4.32. Found: C, 53.99; H, 6.08; N, 4.09.

**trans,trans-2-(2,2-Dimethylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8h):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.80–0.99 (m, 15H), 1.10–1.37 (m, 8H), 1.43–1.58 (m, 4H), 1.77–1.97 (m, 2H), 3.48–3.12 (m, 5H), 3.60–3.69 (m, 1H), 3.75–3.86 (m, 1H), 3.95–4.16 (m, 2H), 4.28–4.4 (m, 2H), 5.94 (s, 2H), 6.74 (d,  $J$  = 8.1 Hz, 1H), 6.8 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.87 (d,  $J$  = 1.8 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  503. Anal. Calcd for  $\text{C}_{29}\text{H}_{48}\text{N}_2\text{O}_5 \cdot 1.05\text{TFA}$ : C, 60.01; H, 7.62; N, 4.50. Found: C, 60.21; H, 7.37; N, 4.33.

**trans,trans-2-(4-Methyl-3-pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8i):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.93 (t,  $J$  = 7.5 Hz, 3H), 0.96 (t,  $J$  = 7.5 Hz, 3H), 1.24–1.38 (m, 4H), 1.35–1.57 (m, 4H), 1.57 (s, 3H), 1.64 (s, 3H), 1.83–2.16 (m, 4H), 3.10–3.40 (m, 5H), 3.69–3.88 (m, 2H), 3.95–4.10 (m, 1H), 4.19 (d,  $J$  = 15.9 Hz, 1H), 4.26–4.34 (m, 1H), 4.39 (d,  $J$  = 16.2 Hz, 1H), 5.00–5.07 (m, 1H), 5.94 (s, 2H), 6.73 (d,  $J$  = 8.1 Hz, 1H), 6.78 (dd,  $J$  = 1.8, 8.1 Hz, 1H), 6.85 (d,  $J$  = 1.8 Hz, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  487. Anal. Calcd for  $\text{C}_{28}\text{H}_{42}\text{N}_2\text{O}_5 \cdot 0.05\text{H}_2\text{O} \cdot 1.15\text{TFA}$ : C, 58.79; H, 6.90; N, 4.34. Found: C, 58.82; H, 7.05; N, 4.53.

**trans,trans-2-(4-Methyl-4-hexenyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8j):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.87–1.01 (m, 6H), 1.23–1.39 (m, 4H), 1.45–1.60 (m, 4H), 1.54 (brs, 3H), 1.66 (brs, 1H), 1.83–2.20 (m, 6H), 1.96 (q,  $J$  = 7.5 Hz, 2H), 3.16 (brt,  $J$  = 7.8 Hz, 2H), 3.22–3.32 (m, 1H), 3.36 (brtm,  $J$  = 7.5 Hz, 2H), 3.69–3.83 (brm, 2H), 4.05 (brdd,  $J$  = 9.6, 18.0 Hz, 1H), 4.23–4.42 (m, 3H), 4.97–5.06 (brm, 1H), 5.96 (s, 2H), 6.77 (brs, 2H), 6.84 (brs, 1H); MS ( $\text{DCI}/\text{NH}_3$ ) ( $M + \text{H}^+$ ) at  $m/z$  501. Anal. Calcd for  $\text{C}_{29}\text{H}_{44}\text{N}_2\text{O}_5 \cdot 3.80\text{TFA}$ : C, 47.07; H, 5.16; N, 3.00. Found: C, 47.14; H, 4.96; N, 2.97.

**trans,trans-2-(3-Cyclopropylidenepropyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8k):** white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  0.92 (t,  $J$  = 7.5 Hz, 3H), 0.95 (t,  $J$  = 7.5

H<sub>2</sub>, 3H), 1.01 (s, 4H), 1.31 (septet,  $J = 7.5$  Hz, 4H), 1.51 (sextet,  $J = 7.5$  Hz, 4H), 1.95–2.41 (m, 4H), 3.16 (t,  $J = 7.5$  Hz, 2H), 3.24–3.43 (m, 3H), 3.73–3.89 (brm, 2H), 4.03 (dd,  $J = 10.5$ , 19.5 Hz, 1H), 4.21 (d,  $J = 16.5$  Hz, 1H), 4.34 (m, 1H), 4.36 (d,  $J = 18.0$  Hz, 1H), 5.70 (brm, 1H), 5.94 (s, 2H), 6.74 (d,  $J = 8.1$  Hz, 1H), 6.79 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.86 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  485. Anal. Calcd for C<sub>28</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub>·1.80TFA: C, 55.02; H, 6.11; N, 4.06. Found: C, 55.12; H, 6.18; N, 4.13.

**trans,trans-2-[3(E)-Pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8l):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 1.32 (septet,  $J = 7.5$  Hz, 4H), 1.51 (sextet,  $J = 7.5$  Hz, 4H), 1.60 (dd,  $J = 6.0$ , 14.7 Hz, 3H), 1.88–2.21 (m, 4H), 3.10–4.43 (m, 5H), 3.72 (dd,  $J = 10.2$ , 22.8 Hz, 1H), 3.77 (dd,  $J = 12.0$ , 21.0 Hz, 1H), 3.95–4.10 (m, 2H), 4.26 (m, 1H), 4.29 (d,  $J = 16.5$  Hz, 1H), 5.23–5.38 (m, 1H), 5.48 (qd,  $J = 6.0$ , 21.0 Hz, 1H), 5.94 (s, 2H), 6.83 (d,  $J = 8.1$  Hz, 1H), 6.89 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.98 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  473. Anal. Calcd for C<sub>27</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub>·0.85TFA: C, 60.52; H, 7.23; N, 4.92. Found: C, 60.63; H, 7.27; N, 4.88.

**trans,trans-2-[3(Z)-Pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8m):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$  Hz, 3H), 1.20–1.40 (m, 4H), 1.45–1.63 (m, 4H), 1.57 (dd,  $J = 1.5$ , 6.9 Hz, 3H), 1.88–2.24 (m, 4H), 3.15 (td,  $J = 1.8$ , 7.8 Hz, 2H), 3.25–3.42 (m, 3H), 3.68–3.88 (m, 2H), 4.03 (dd,  $J = 9.0$ , 11.1 Hz, 1H), 4.12 (d,  $J = 16.8$  Hz, 1H), 4.32 (brm, 1H), 4.35 (d,  $J = 16.8$  Hz, 1H), 5.29 (brm, 1H), 5.49 (dd,  $J = 6.0$ , 11.1 Hz, 1H), 5.94 (s, 2H), 6.74 (d,  $J = 8.1$  Hz, 1H), 6.81 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.88 (d,  $J = 1.8$  Hz, 1H); MS (APCI) (M + H)<sup>+</sup> at  $m/z$  473. Anal. Calcd for C<sub>27</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub>·1.10TFA·1.05AcOH: C, 57.10; H, 6.96; N, 4.25. Found: C, 57.17; H, 6.70; N, 3.97.

**trans,trans-2-(4-Pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8n):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 1.24–1.38 (m, 4H), 1.42–1.65 (m, 6H), 1.80–2.03 (m, 2H), 2.09 (q,  $J = 7.0$  Hz, 2H), 3.12–3.44 (m, 5H), 3.72 (t,  $J = 11.3$  Hz, 1H), 3.84 (t,  $J = 10.5$  Hz, 1H), 3.98 (d,  $J = 12.0$  Hz, 1H), 4.03 (d,  $J = 16.8$  Hz, 1H), 4.30–4.40 (m, 1H), 4.37 (d,  $J = 16.8$  Hz, 1H), 4.96 (m, 1H), 5.01 (dd,  $J = 2.4$ , 11.7 Hz, 1H), 5.71 (tdd,  $J = 3.3$ , 6.9, 18.0 Hz, 1H), 5.95 (s, 2H), 6.74 (d,  $J = 8.1$  Hz, 1H), 6.81 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  473. Anal. Calcd for C<sub>27</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub>·1.05TFA: C, 59.01; H, 6.99; N, 4.73. Found: C, 58.91; H, 6.72; N, 4.50.

**trans,trans-2-(4-Methyl-4-pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8o):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 1.31 (septet,  $J = 7.5$  Hz, 4H), 1.53 (sextet,  $J = 7.5$  Hz, 4H), 1.67 (s, 3H), 1.87–2.0 (m, 2H), 2.04 (t,  $J = 7.2$  Hz, 2H), 3.17 (brtd,  $J = 3.0$ , 9.0 Hz, 2H), 3.37 (dd,  $J = 8.4$ , 18.0 Hz, 2H), 3.38 (dd,  $J = 6.0$ , 13.8 Hz, 2H), 3.75 (t,  $J = 11.4$  Hz, 1H), 3.86 (t,  $J = 9.0$  Hz, 1H), 4.04 (dd,  $J = 11.1$ , 21.0 Hz, 1H), 4.11 (d,  $J = 16.8$  Hz, 1H), 4.37 (d,  $J = 16.8$  Hz, 1H), 4.40 (brm, 1H), 4.64 (s, 1H), 4.72 (s, 1H), 5.95 (s, 2H), 6.75 (d,  $J = 8.1$  Hz, 1H), 6.82 (d,  $J = 1.8$ , 8.1 Hz, 1H), 6.89 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  487. Anal. Calcd for C<sub>28</sub>H<sub>42</sub>N<sub>2</sub>O<sub>5</sub>·1.30TFA: C, 57.89; H, 6.87; N, 4.41. Found: C, 57.97; H, 6.84; N, 4.48.

**trans,trans-2-[3-Methyl-3(E)-pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8p):** white amorphous solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.97 (t,  $J = 7.5$  Hz, 3H), 1.22–1.40 (m, 5H), 1.44–1.61 (m, 8H), 1.82 (brm, 1H), 2.02 (m, 3H), 3.05–3.30 (m, 5H), 3.38 (m, 1H), 3.55 (brm, 2H), 3.85 (m, 3H), 4.12 (brd,  $J = 15.0$  Hz, 1H), 5.21 (dd,  $J = 6.0$ , 12.0 Hz, 1H), 5.93 (s, 2H), 6.73 (d,  $J = 8.1$  Hz, 1H), 6.78 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.88 (d,  $J = 1.8$  Hz, 1H); MS

(DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  487. Anal. Calcd for C<sub>28</sub>H<sub>42</sub>N<sub>2</sub>O<sub>5</sub>·0.70TFA: C, 62.34; H, 7.60; N, 4.95. Found: C, 62.49; H, 7.43; N, 4.73.

**trans,trans-2-(2,2,4-Trimethyl-3-pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8q):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.94 (t,  $J = 7.5$  Hz, 3H), 1.11 (s, 3H), 1.13 (s, 3H), 1.24–1.37 (m, 4H), 1.46–1.59 (m, 4H), 1.61 (d,  $J = 1.2$  Hz, 3H), 1.69 (d,  $J = 1.2$  Hz, 3H), 2.04–2.11 (m, 2H), 3.10–3.20 (m, 2H), 3.30–3.39 (m, 3H), 3.67–3.82 (m, 2H), 3.95–4.08 (m, 1H), 4.32 (m, 2H), 4.37–4.47 (m, 1H), 4.99 (s, 1H), 5.95 (s, 2H), 6.73 (d,  $J = 8.1$  Hz, 1H), 6.78 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.84 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  515. Anal. Calcd for C<sub>30</sub>H<sub>46</sub>N<sub>2</sub>O<sub>5</sub>·1.05TFA: C, 60.77; H, 7.48; N, 4.42. Found: C, 60.83; H, 7.20; N, 4.43.

**trans,trans-2-[2S,3R,4S]-2-[2,2-Dimethyl-3(E)-pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8r):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 0.98 (s, 3H), 1.00 (s, 3H), 1.32 (septet,  $J = 7.5$  Hz, 4H), 1.54 (sextet,  $J = 7.5$  Hz, 4H), 1.60 (d,  $J = 5.7$  Hz, 3H), 1.80–1.94 (m, 1H), 1.96–2.06 (m, 1H), 3.12–3.32 (m, 3H), 3.35 (td,  $J = 3.0$ , 9.6 Hz, 2H), 3.60–3.69 (m, 2H), 3.87–4.20 (m, 3H), 4.14 (brd,  $J = 15.0$  Hz, 1H), 5.30 (d,  $J = 15.6$  Hz, 1H), 5.39 (qd,  $J = 6.0$ , 15.6 Hz, 1H), 5.94 (s, 2H), 6.74 (d,  $J = 8.1$  Hz, 1H), 6.82 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.88 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  501. Anal. Calcd for C<sub>28</sub>H<sub>44</sub>N<sub>2</sub>O<sub>5</sub>·0.70TFA: C, 62.90; H, 7.76; N, 4.83. Found: C, 62.72; H, 7.83; N, 4.82.

**trans,trans-2-[2-(1,3-Dioxol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8a):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$  Hz, 3H), 1.23–1.38 (m, 4H), 1.52 (sextet,  $J = 7.5$  Hz, 4H), 1.85–1.95 (m, 2H), 2.02–2.17 (m, 2H), 3.18 (dd,  $J = 6.0$ , 9.0 Hz, 2H), 3.30 (dd,  $J = 9.0$ , 18.0 Hz, 2H), 3.35 (m, 1H), 3.79 (dd,  $J = 3.6$ , 6.9 Hz, 1H), 3.83–3.88 (m, 3H), 3.97 (dd,  $J = 4.8$ , 6.0 Hz, 1H), 4.05 (q,  $J = 9.6$  Hz, 2H), 4.30–4.40 (m, 1H), 4.37 (s, 2H), 4.87 (t,  $J = 4.1$  Hz, 1H), 5.94 (s, 2H), 6.73 (d,  $J = 8.1$  Hz, 1H), 6.79 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (APCI) (M + H)<sup>+</sup> at  $m/z$  505. Anal. Calcd for C<sub>27</sub>H<sub>40</sub>N<sub>2</sub>O<sub>7</sub>·1.20TFA: C, 55.05; H, 6.47; N, 4.37. Found: C, 55.12; H, 6.44; N, 4.27.

**trans,trans-2-[2-(4,5-Dimethyl-1,3-dioxol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8b):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz, mixture of diastereomers)  $\delta$  0.89–0.99 (m, 6H), 1.15 (t,  $J = 7.5$  Hz, 3H), 1.20–1.38 (m, 7H), 1.44–1.60 (m, 4H), 1.75–1.95 (m, 2H), 1.97–2.26 (m, 2H), 3.13–3.40 (m, 4H), 3.62–3.70 (m, 1H), 3.70–3.88 (m, 2H), 4.05 (m, 1H), 4.16 (m, 1H), 4.25–4.45 (m, 4H), 4.88 (t,  $J = 4.4$  Hz) and 5.06 (td,  $J = 1.2$ , 4.4 Hz, 1H in total), 5.96 (s, 2H), 6.77 (brm, 2H), 6.83 (brs, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  533. Anal. Calcd for C<sub>29</sub>H<sub>44</sub>N<sub>2</sub>O<sub>7</sub>·1.35TFA: C, 55.45; H, 6.66; N, 4.08. Found: C, 55.47; H, 6.47; N, 4.03.

**trans,trans-2-[2-(1,3-Dioxan-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8c):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.90 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 1.23–1.41 (m, 6H), 1.42–1.62 (m, 6H), 1.62–1.75 (m, 2H), 2.66 (dd,  $J = 6.9$ , 8.4 Hz, 1H), 2.83–2.94 (m, 3H), 3.07 (td,  $J = 7.8$ , 15.0 Hz, 1H), 3.16 (dd,  $J = 3.9$ , 9.6 Hz, 2H), 3.38–3.69 (m, 3H), 3.72 (d,  $J = 8.4$  Hz, 1H), 3.76 (dd,  $J = 3.0$ , 10.2 Hz, 2H), 4.07 (dd,  $J = 4.2$ , 11.1 Hz, 2H), 4.52 (t,  $J = 4.5$  Hz, 1H), 5.91 (dd,  $J = 2.0$ , 4.0 Hz, 2H), 6.68 (d,  $J = 8.1$  Hz, 1H), 6.73 (dd,  $J = 1.8$ , 8.1 Hz, 1H), 6.86 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) (M + H)<sup>+</sup> at  $m/z$  519. Anal. Calcd for C<sub>28</sub>H<sub>42</sub>N<sub>2</sub>O<sub>7</sub>·1.50TFA: C, 53.99; H, 6.36; N, 4.06. Found: C, 54.06; H, 6.50; N, 3.99.

**trans,trans-2-[3-(1,3-Dioxol-2-yl)propyl]-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di-n-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8d):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$

Hz, 3H), 1.32 (septet,  $J = 7.5$  Hz, 4H), 1.52 (sextet,  $J = 7.5$  Hz, 6H), 1.65–1.75 (m, 2H), 1.85–2.12 (m, 2H), 3.17 (dd,  $J = 6.3, 9.3$  Hz, 2H), 3.21–3.45 (m, 3H), 3.78 (dd,  $J = 3.6, 6.9$  Hz, 1H), 3.80–3.97 (m, 5H), 4.03 (q,  $J = 9.7$  Hz, 1H), 4.16 (d,  $J = 16.5$  Hz, 1H), 4.33 (m, 1H), 4.37 (d,  $J = 16.5$  Hz, 1H), 4.82 (t,  $J = 4.5$  Hz, 1H), 5.95 (s, 2H), 6.75 (d,  $J = 8.1$  Hz, 1H), 6.81 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.88 (d,  $J = 1.8$  Hz, 1H); MS (APCI) ( $M + H$ )<sup>+</sup> at  $m/z$  519. Anal. Calcd for  $C_{28}H_{42}N_2O_7 \cdot 1.50TFA$ : C, 53.99; H, 6.36; N, 4.06. Found: C, 54.06; H, 6.26; N, 4.05.

**trans,trans-2-[2-(1,3-Dithia)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9e):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$  Hz, 3H), 1.24–1.40 (m, 4H), 1.45–1.61 (m, 4H), 1.89 (q,  $J = 7.4$  Hz, 2H), 1.97–2.10 (m, 1H), 2.15–2.25 (m, 1H), 3.13–3.45 (m, 9H), 3.75–3.93 (m, 2H), 4.03 (dd,  $J = 9.6, 18.9$  Hz, 1H), 4.25 (d,  $J = 15.9$  Hz, 1H), 4.28–4.39 (m, 1H), 4.44 (d,  $J = 15.9$  Hz, 1H), 4.50 (t,  $J = 6.5$  Hz, 1H), 5.96 (s, 2H), 6.76 (d,  $J = 8.1$  Hz, 1H), 6.79 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (APCI) ( $M + H$ )<sup>+</sup> at  $m/z$  537. Anal. Calcd for  $C_{27}H_{40}N_2O_5S_2 \cdot 1.15TFA$ : C, 52.69; H, 6.21; N, 4.19. Found: C, 52.68; H, 5.97; N, 4.00.

**trans,trans-2-[2,2-Dimethyl-2-(1,3-dioxol-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9f):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.82–1.00 (m, 12H), 1.24–1.40 (m, 4H), 1.43–1.64 (m, 5H), 1.76–1.84 (m, 1H), 2.93–3.00 (m, 1H), 3.15–3.47 (m, 6H), 3.60–3.70 (m, 3H), 3.74–3.95 (m, 5H), 4.48 (s, 1H), 5.94 (m, 2H), 6.72 (d,  $J = 8.1$  Hz, 1H), 6.83 (dd,  $J = 1.8, 8.0$  Hz, 1H), 6.94 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  533. Anal. Calcd for  $C_{28}H_{44}N_2O_7 \cdot 1.10TFA \cdot 0.2H_2O$ : C, 56.63; H, 6.93; N, 4.23. Found: C, 56.60; H, 6.96; N, 4.25.

**trans,trans-2-[2-(2-Tetrahydropyranyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9g):** an amorphous white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz, 1:1 mixture of two diastereomers)  $\delta$  0.89 (t,  $J = 7.5$  Hz, 3H), 0.91 (t,  $J = 7.5$  Hz, 3H), 1.30 (septet, 1H), 1.42–1.66 (m, 8H), 1.71 (brm, 1H), 1.85 (brm, 1H), 1.96–2.23 (brm, 2H), 3.10–3.29 (m, 4H), 3.29–3.52 (m, 3H), 3.54–3.81 (m, 3H), 4.01 (q,  $J = 9.0$  Hz, 1H), 4.12–4.25 (m, 2H), 4.43 (d,  $J = 9.0$  Hz, 1H), 4.50 (d,  $J = 2.7$  Hz, 1H), 5.94 (s) and 5.95 (s, 2H in total), 6.76 (s, 2H), 6.81 (s, 1H); MS (APCI) ( $M + H$ )<sup>+</sup> at  $m/z$  517. Anal. Calcd for  $C_{28}H_{44}N_2O_6 \cdot 1.40TFA$ : C, 56.48; H, 6.77; N, 4.14. Found: C, 56.46; H, 6.99; N, 3.83.

**trans,trans-2-[2-(4-Tetrahydro-2H-pyranyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9h):** an amorphous white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.90 (t,  $J = 7.5$  Hz, 3H), 0.97 (t,  $J = 7.5$  Hz, 3H), 1.17–1.42 (m, 8H), 1.42–1.66 (m, 8H), 1.66–1.81 (m, 1H), 2.73 (t,  $J = 7.2$  Hz, 1H), 3.06 (dd,  $J = 9.6, 18.6$  Hz, 2H), 3.11–3.60 (m, 9H), 3.68 (d,  $J = 13.5$  Hz, 1H), 3.94 (dd,  $J = 2.7, 12.0$  Hz, 2H), 5.91 (s, 2H), 6.71 (d,  $J = 8.1$  Hz, 1H), 6.78 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  517. Anal. Calcd for  $C_{28}H_{44}N_2O_6$ : C, 67.42; H, 8.58; N, 5.42. Found: C, 67.39; H, 8.72; N, 5.30.

**trans,trans-2-[2-(2-Furfuryl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9i):** an amorphous white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.89–0.96 (m, 6H), 1.21–1.38 (m, 4H), 1.42–1.57 (m, 4H), 2.19 (m, 1H), 2.31 (m, 1H), 2.63–2.85 (m, 2H), 3.08–3.39 (m, 5H), 3.69 (d,  $J = 10$  Hz, 2H), 3.90–4.01 (m, 2H), 4.12–4.21 (m, 2H), 5.92 (s, 2H), 6.03 (d,  $J = 3.0$  Hz, 1H), 6.24 (dd,  $J = 2.0, 3.0$  Hz, 1H), 6.75 (d,  $J = 8.1$  Hz, 1H), 6.79 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.88 (d,  $J = 1.8$  Hz, 1H), 7.25 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  499. Anal. Calcd for  $C_{28}H_{38}N_2O_6 \cdot 0.80TFA \cdot 0.15H_2O$ : C, 60.00; H, 6.65; N, 4.73. Found: C, 60.00; H, 6.69; N, 4.55.

**trans,trans-2-[2-(3-Furfuryl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9j):** an amorphous white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.87–0.96 (m, 6H), 1.21–1.39 (m,

4H), 1.42–1.60 (m, 4H), 1.90 (m, 1H), 2.03 (m, 1H), 2.39–2.60 (m, 2H), 2.96 (m, 1H), 3.15–3.50 (m, 8H), 3.65–3.85 (m, 2H), 5.91 (s, 2H), 6.24 (s, 1H), 6.70 (d,  $J = 8.1$  Hz, 1H), 6.79 (d,  $J = 8.1$  Hz, 1H), 6.90 (s, 1H), 7.20 (s, 1H), 7.32 (s, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  499. Anal. Calcd for  $C_{28}H_{38}N_2O_6 \cdot 0.20TFA$ : C, 65.42; H, 7.38; N, 5.37. Found: C, 65.25; H, 7.41; N, 5.27.

**trans,trans-2-[2-(2-Tetrahydrofuran)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9k):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz, a 1:1 mixture of two diastereomers)  $\delta$  0.86–1.00 (m, 6H), 1.22–1.40 (m, 4H), 1.40–1.62 (m, 6H), 1.63–1.77 (m, 1H), 1.80–2.07 (m, 5H), 2.95–3.62 (m, 8H), 3.68–4.20 (m, 6H), 5.92 (s, 2H), 6.72 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.80 (dm,  $J = 8.1$  Hz, 1H), 6.86 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  503. Anal. Calcd for  $C_{28}H_{42}N_2O_6 \cdot 0.45TFA$ : C, 62.66; H, 7.72; N, 5.06. Found: C, 62.62; H, 7.53; N, 4.93.

**trans,trans-2-[2-(1,3-Oxazol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9l):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.91 (t,  $J = 7.5$  Hz, 3H), 0.93 (t,  $J = 7.5$  Hz, 3H), 1.22–1.36 (m, 4H), 1.43–1.60 (m, 4H), 2.35–2.60 (brm, 2H), 3.08–3.35 (m, 7H), 3.65 (dd,  $J = 9.0, 12.3$  Hz, 1H), 3.88 (t,  $J = 11.3$  Hz, 1H), 4.07 (dd,  $J = 9.9, 20.0$  Hz, 1H), 4.40 (brm, 2H), 4.57 (d,  $J = 16.5$  Hz, 1H), 5.92 (s, 2H), 6.76 (m, 3H), 7.08 (s, 1H), 7.64 (s, 1H); MS (ESI) ( $M + H$ )<sup>+</sup> at  $m/z$  500. Anal. Calcd for  $C_{27}H_{37}N_3O_6 \cdot 1.70TFA$ : C, 52.66; H, 5.63; N, 6.06. Found: C, 52.75; H, 5.80; N, 6.09.

**trans,trans-2-[2-(4,5-Dimethyl-1,3-oxazol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9m):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.89 (t,  $J = 7.5$  Hz, 3H), 0.92 (t,  $J = 7.5$  Hz, 3H), 1.21–1.37 (m, 4H), 1.43–1.57 (m, 4H), 2.11 (s, 3H), 2.21 (s, 3H), 2.31–2.47 (m, 2H), 2.95–3.41 (m, 7H), 3.62 (dd,  $J = 9.0, 12.3$  Hz, 1H), 3.83 (t,  $J = 12.3$  Hz, 1H), 4.10 (dd,  $J = 10.2, 19.8$  Hz, 1H), 4.30–4.40 (brm, 1H), 4.37 (d,  $J = 16.5$  Hz, 1H), 4.57 (d,  $J = 16.5$  Hz, 1H), 5.91 (s, 2H), 6.71 (dd,  $J = 1.8, 8.1$  Hz, 2H), 6.76 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  528. Anal. Calcd for  $C_{28}H_{41}N_3O_6 \cdot 1.05TFA$ : C, 57.70; H, 6.55; N, 6.49. Found: C, 57.89; H, 6.33; N, 6.41.

**trans,trans-2-[2-(*N*-Methylpyrrol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9n):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.93 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 1.22–1.37 (m, 4H), 1.42–1.56 (m, 4H), 2.11–2.36 (m, 2H), 2.55–2.80 (m, 2H), 3.04–3.15 (m, 2H), 3.26–3.40 (m, 3H), 3.50 (s, 3H), 3.74 (d,  $J = 9.6$  Hz, 2H), 3.88 (d,  $J = 16.5$  Hz, 1H), 4.02 (dd,  $J = 9.0, 18.6$  Hz, 1H), 4.21 (d,  $J = 16.5$  Hz, 1H), 4.32–4.43 (m, 1H), 5.86–5.89 (m, 1H), 5.91 (s, 2H), 6.00 (t,  $J = 4.5$  Hz, 1H), 6.52 (t,  $J = 3.0$  Hz, 1H), 6.72 (d,  $J = 8.1$  Hz, 1H), 6.80 (dd,  $J = 1.8, 8.0$  Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  512. Anal. Calcd for  $C_{28}H_{41}N_3O_5 \cdot 1.25TFA$ : C, 57.83; H, 6.51; N, 6.42. Found: C, 57.68; H, 6.69; N, 6.49.

**trans,trans-2-[2-(2-Pyridyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9o):** an amorphous white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.91 (t,  $J = 7.5$  Hz, 6H), 1.22–1.37 (m, 4H), 1.43–1.60 (m, 4H), 2.51 (brs, 2H), 3.13–3.46 (m, 7H), 3.62 (dd,  $J = 9.6, 13.8$  Hz, 1H), 3.84 (t,  $J = 12.6$  Hz, 1H), 4.04 (dd,  $J = 10.5, 20.1$  Hz, 1H), 4.18–4.29 (m, 1H), 4.45 (s, 2H), 5.91 (s, 2H), 6.66 (d,  $J = 8.1$  Hz, 1H), 6.75 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.80 (d,  $J = 1.8$  Hz, 1H), 7.51 (t,  $J = 6.9$  Hz, 1H), 7.70 (d,  $J = 9.0$  Hz, 1H), 8.06 (t,  $J = 6.9$  Hz, 1H), 8.65 (d,  $J = 6.0$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  510. Anal. Calcd for  $C_{28}H_{35}N_3O_5 \cdot 1.75TFA$ : C, 55.04; H, 5.79; N, 5.92. Found: C, 55.08; H, 5.64; N, 5.81.

**trans,trans-2-[2-(1-Pyrazolyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9p):** an amorphous solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.91 (t,  $J = 7.5$  Hz, 6H), 1.21–1.38 (m, 4H), 1.42–1.59 (m, 4H), 2.43–2.69 (m, 2H), 3.12–3.36 (m, 5H), 3.40–3.61 (t,  $J = 10.5$  Hz, 1H), 3.72–3.83 (t,  $J = 10.5$  Hz, 1H), 3.98–4.55 (m, 6H), 5.91 (s, 2H), 6.28 (t,  $J = 3.0$  Hz, 1H),

6.67 (d,  $J = 8.1$  Hz, 1H), 6.75 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.81 (d,  $J = 1.8$  Hz, 1H), 7.50 (d,  $J = 3.0$  Hz, 1H), 7.56 (d,  $J = 3.0$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  499. Anal. Calcd for C<sub>27</sub>H<sub>38</sub>N<sub>4</sub>O<sub>5</sub>·0.75TFA: C, 58.60; H, 6.69; N, 9.59. Found: C, 58.53; H, 6.45; N, 9.67.

**trans,trans-2-[2-(Succinimido)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9g):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$  Hz, 3H), 1.23–1.41 (m, 4H), 1.44–1.63 (m, 4H), 2.10–2.25 (m, 1H), 2.37–2.48 (m, 1H), 2.69 (s, 4H), 3.20 (t,  $J = 7.8$  Hz, 2H), 3.26–3.40 (m, 3H), 3.50–3.73 (m, 3H), 3.81 (t,  $J = 10.5$  Hz, 1H), 3.94 (dd,  $J = 9.0, 19.2$  Hz, 1H), 4.09–4.21 (m, 2H), 4.33 (d,  $J = 16.8$  Hz, 1H), 5.92 (s, 2H), 6.72 (d,  $J = 8.1$  Hz, 1H), 6.81 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  530. Anal. Calcd for C<sub>28</sub>H<sub>39</sub>N<sub>3</sub>O<sub>7</sub>·1.05TFA: C, 55.68; H, 6.62; N, 6.47. Found: C, 55.77; H, 6.02; N, 6.19.

**trans,trans-2-[2-(Propylsultam-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9r):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$  Hz, 3H), 1.23–1.38 (m, 4H), 1.44–1.61 (m, 4H), 2.10–2.42 (m, 4H), 3.02–3.26 (m, 7H), 3.26–3.44 (m, 5H), 3.73 (brt,  $J = 10.0$  Hz, 1H), 3.90 (q,  $J = 8.0$  Hz, 1H), 3.93–4.10 (m, 2H), 4.26 (d,  $J = 15.3$  Hz, 1H), 5.94 (s, 2H), 6.73 (d,  $J = 8.1$  Hz, 1H), 6.81 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.92 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  552. Anal. Calcd for C<sub>27</sub>H<sub>41</sub>N<sub>3</sub>O<sub>7</sub>S<sub>2</sub>·0.60TFA: C, 54.62; H, 6.76; N, 6.78. Found: C, 54.74; H, 6.74; N, 6.69.

**trans,trans-2-[2-(2-Oxopiperidin-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9s):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.91 (t,  $J = 7.5$  Hz, 3H), 0.93 (t,  $J = 7.5$  Hz, 3H), 1.22–1.38 (m, 4H), 1.42–1.60 (m, 4H), 1.70–1.87 (m, 4H), 2.10–2.23 (brm, 1H), 2.23–2.50 (m, 3H), 3.05 (t,  $J = 9.0$  Hz, 1H), 3.19–3.40 (m, 7H), 3.40–3.60 (m, 2H), 3.61–3.74 (m, 1H), 3.75–3.99 (m, 3H), 4.22 (brs, 1H), 5.92 (m, 2H), 6.72 (d,  $J = 8.1$  Hz, 1H), 6.78 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  530. Anal. Calcd for C<sub>29</sub>H<sub>43</sub>N<sub>3</sub>O<sub>6</sub>·0.70TFA: C, 59.91; H, 7.23; N, 6.89. Found: C, 59.92; H, 7.23; N, 6.80.

**trans,trans-2-[2-(2-Oxopyridin-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9t):** white powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.89 (t,  $J = 7.5$  Hz, 3H), 0.94 (t,  $J = 7.5$  Hz, 3H), 1.21–1.39 (m, 4H), 1.41–1.61 (m, 4H), 2.38 (brm, 2H), 3.09–3.24 (m, 3H), 3.32 (t,  $J = 8.4$  Hz, 2H), 3.54 (brm, 1H), 3.73 (t,  $J = 11.4$  Hz, 1H), 3.88 (dd,  $J = 8.4, 18.3$  Hz, 1H), 3.94–4.10 (m, 2H), 4.15–4.30 (m, 3H), 5.96 (s, 2H), 6.31 (td,  $J = 1.5, 6.9$  Hz, 1H), 6.58 (d,  $J = 9.0$  Hz, 1H), 6.72 (d,  $J = 8.1$  Hz, 1H), 6.77 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.84 (d,  $J = 1.8$  Hz, 1H), 7.40 (td,  $J = 1.8, 6.3$  Hz, 1H), 7.60 (dd,  $J = 1.5, 6.0$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  526. Anal. Calcd for C<sub>29</sub>H<sub>39</sub>N<sub>3</sub>O<sub>6</sub>·0.70TFA: C, 60.31; H, 6.61; N, 6.94. Found: C, 60.17; H, 6.59; N, 6.91.

**trans,trans-2-[2-(2-Oxopyrrolidin-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9u):** an amorphous white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.91 (t,  $J = 7.5$  Hz, 3H), 0.94 (t,  $J = 7.5$  Hz, 3H), 1.23–1.38 (m, 4H), 1.44–1.60 (m, 4H), 2.05 (t,  $J = 6.9$  Hz, 2H), 2.12–2.25 (m, 2H), 2.38 (td,  $J = 4.2, 8.4$  Hz, 2H), 2.47–2.61 (m, 1H), 3.17 (dd,  $J = 6.0, 8.7$  Hz, 2H), 3.24 (t,  $J = 9.0$  Hz, 1H), 3.32 (t,  $J = 7.8$  Hz, 2H), 3.38–3.48 (m, 3H), 3.52 (t,  $J = 9.0$  Hz, 1H), 3.66 (t,  $J = 6.9$  Hz, 1H), 3.96 (m, 2H), 4.14 (m, 1H), 4.38 (brs, 2H), 5.93 (s, 2H), 6.74 (d,  $J = 8.1$  Hz, 1H), 6.89 (dd,  $J = 1.8, 8.1$  Hz, 1H), 6.87 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  516. Anal. Calcd for C<sub>28</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>·1.40TFA: C, 54.78; H, 6.33; N, 6.22. Found: C, 54.69; H, 6.33; N, 6.14.

**trans,trans-[2S,3R,4S]-2-[2,2-Dimethylpentyl]-4-(7-methoxy-1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (10a):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.81 (s, 3H), 0.84 (s, 3H),

0.86 (t,  $J = 7.5$  Hz, 3H), 0.93 (t,  $J = 7.5$  Hz, 3H), 0.96 (t,  $J = 7.5$  Hz, 3H), 1.09–1.38 (m, 8H), 1.45–1.59 (m, 4H), 1.84–2.00 (m, 2H), 3.15 (dd,  $J = 6.9, 10.0$  Hz, 2H), 3.30–3.42 (m, 3H), 3.72 (t,  $J = 10.5$  Hz, 1H), 3.86 (t,  $J = 10.5$  Hz, 1H), 3.88 (s, 3H), 4.02 (q,  $J = 10.0$  Hz, 1H), 4.12 (d,  $J = 16.8$  Hz, 1H), 4.29 (d,  $J = 16.8$  Hz, 1H), 4.41 (brm, 1H), 5.94 (s, 2H), 6.52 (d,  $J = 1.8$  Hz, 1H), 6.67 (d,  $J = 1.8$  Hz, 1H); MS (ESI) ( $M + H$ )<sup>+</sup> at  $m/z$  533. Anal. Calcd for C<sub>30</sub>H<sub>48</sub>N<sub>2</sub>O<sub>6</sub>·0.7TFA: C, 61.57; H, 8.01; N, 4.57. Found: C, 61.59; H, 8.20; N, 4.63.

**trans,trans-[2S,3R,4S]-2-[2,2-Dimethyl-3(*E*)-pentenyl]-4-(7-methoxy-1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (10b):** white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.92 (t,  $J = 7.5$  Hz, 3H), 0.95 (t,  $J = 7.5$  Hz, 3H), 0.97 (s, 3H), 0.99 (s, 3H), 1.24–1.40 (m, 4H), 1.46–1.60 (m, 4H), 1.60 (d,  $J = 5.4$  Hz, 3H), 1.92 (dd,  $J = 6.6, 15.0$  Hz, 1H), 2.04 (d,  $J = 15.0$  Hz, 1H), 3.17 (td,  $J = 3.0, 11.4$  Hz, 2H), 3.25–3.40 (m, 3H), 3.55–3.75 (m, 2H), 3.87 (s, 3H), 3.99 (q,  $J = 9.0$  Hz, 1H), 4.11–4.30 (m, 3H), 5.29 (d,  $J = 15.6$  Hz, 1H), 5.38 (qd,  $J = 6.0, 15.6$  Hz, 1H), 5.94 (s, 2H), 6.50 (d,  $J = 1.8$  Hz, 1H), 6.63 (d,  $J = 1.8$  Hz, 1H); MS (DCI/NH<sub>3</sub>) ( $M + H$ )<sup>+</sup> at  $m/z$  531. Anal. Calcd for C<sub>30</sub>H<sub>48</sub>N<sub>2</sub>O<sub>6</sub>·0.80TFA: C, 61.03; H, 7.58; N, 4.50. Found: C, 61.04; H, 7.65; N, 4.46.

**Receptor Binding Assays.** All samples were kept at 4 °C throughout the process of membrane isolation. MMQ cells (prolactin secreting rat pituitary cells known to contain ET<sub>A</sub> receptors), porcine cerebellar tissues (known to contain ET<sub>B</sub> receptors), or Chinese hamster ovary cells (CHO) permanently transfected with the human ET<sub>A</sub> or ET<sub>B</sub> receptor are homogenized in 25 mL of 10 mM Hepes (pH 7.4) containing 0.25 M sucrose and a protease inhibitor cocktail [50 mM EDTA, 0.1 mM PMSF, and 5  $\mu$ g/mL Pepstatin A, and 0.025% Bacitracin] using a micro ultrasonic cell disruptor (Kontes). The mixture was centrifuged at 1000g for 10 min. The supernatant was collected and centrifuged at 60000g for 60 min. The precipitate was resuspended in 20 mM Tris, pH 7.4, containing protease inhibitor cocktail and centrifuged again. The final membrane pellet was resuspended in 20 mM Tris, pH 7.4, containing protease inhibitors and stored at –80 °C until used. Protein content was determined by the Bio-Rad dye-binding protein assay.

Binding assays were performed in 96-well microtiter plates pretreated with 0.1% BSA. Membranes were diluted ~100-fold in buffer B (20 mM Tris, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, pH 7.4, with 0.2% BSA, 0.1 mM PMSF, 5  $\mu$ g/mL Pepstatin A, 0.025% bacitracin, and 50 mM EDTA) to a final concentration of 0.2 mg/mL of protein. In competition binding studies, membranes (0.02 mg) were incubated with 0.1 nM [<sup>125</sup>I]ET-1 (for ET<sub>A</sub> assay in MMQ or CHO cells) or [<sup>125</sup>I]ET-3 (for ET<sub>B</sub> assay in porcine cerebellum or CHO cells) in buffer B (final volume: 0.2 mL) in the presence of increasing concentrations of the test compound for 3 h at 25 °C. After incubation, unbound ligand was separated from bound ligand by a vacuum filtration method using glass-fiber filter strips in PHD cell harvesters (Cambridge Technology, Inc., MA), washing the filter strips three times with saline (1 mL). Nonspecific binding was determined in the presence of 1  $\mu$ M unlabeled ET-1. IC<sub>50</sub> values are calculated using an average of at least two separate determinations.

**Phosphoinositol Hydrolysis Assays.** ET<sub>A</sub>. MMQ cells (0.4 × 10<sup>6</sup> cells/mL) were labeled with 10  $\mu$ Ci/mL of [<sup>3</sup>H]-myo-inositol in RPMI for 16 h. The cells were washed with PBS and then incubated with buffer A (140 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 0.8 mM MgSO<sub>4</sub>, 5 mM glucose, 25 mM Hepes, pH 7.4) containing protease inhibitors and 10 mM LiCl for 60 min. The cells were incubated with test compounds for 5 min and then challenged with 1 nM ET-1. ET-1 challenge was terminated by the addition of 1.5 mL of 1:2 (v/v) chloroform-methanol. Total inositol phosphates were extracted after adding chloroform and water to give final proportions of 1:1:0.9 (v/v/v) chloroform-methanol-water of as described by Berridge.<sup>29</sup> The upper aqueous phase (1 mL) was retained, and a small portion (100  $\mu$ L) was counted. The rest of the

aqueous sample was analyzed by batch chromatography using anion-exchange resin AG1-X8 (Bio-Rad).

**ET<sub>B</sub>.** Chinese hamster ovary cells (CHO) permanently transfected with the human ET<sub>B</sub> receptor were grown to confluence in 24-well tissue culture plates and labeled with 5  $\mu$ Ci/well of [<sup>3</sup>H]myo-inositol in F-12 media + 10% FBS + 1  $\times$  P/S/F. The adherent cells were washed gently with PBS and then incubated in 200  $\mu$ L of buffer A containing protease inhibitors and 10 mM LiCl for 60 min at 37 °C in a CO<sub>2</sub> incubator. Test compounds were then added followed by the addition of 1 nM ET-1, and the mixtures were incubated for 30 min at 37 °C. The cells were then solubilized by the addition of 50  $\mu$ L of 1 N NaOH and then neutralized by the addition of 50  $\mu$ L of 1 N HCl. The solubilized cell suspension was transferred to glass tubes and extracted by the addition of 1.5 mL of 1:2 (v/v) chloroform-methanol. Total inositol phosphates were extracted and analyzed by batch chromatography on anion-exchange resin as above. All IC<sub>50</sub> values are calculated using an average of at least two separate determinations.

**Pharmacokinetic Analysis.** The pharmacokinetic behavior of compounds were evaluated in male Sprague-Dawley rats. Briefly, the test compound was prepared as a 10 mg/mL solution in an ethanol-propylene glycol-D5W (20:30:50, by volume) vehicle containing 1 molar equiv of sodium hydroxide. Groups of rats ( $n = 4$  per group) received either a 10 mg/kg (1 mL/kg) intravenous dose administered as a slow bolus in the jugular vein or a 10 mg/kg (1 mL/kg) oral dose administered by gavage. Heparinized blood samples (~0.4 mL/sample) were obtained from a tail vein of each rat 0.1 (iv only), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 h after dosing. The samples were analyzed by reverse phase HPLC following liquid-liquid extraction from the plasma. Initial estimates of the pharmacokinetic parameters (e.g., the maximum concentration  $C_{max}$  for NONLIN84<sup>30</sup> were obtained with the program CSTRIP.<sup>31</sup> Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant ( $\beta$ ) was utilized in the extrapolation of the AUC from 12 h to infinity to provide an AUC<sub>0- $\infty$</sub>  value and in the calculation of  $T_{1/2}$  values. Assuming dose proportionality and correcting for the differences in dosing, a comparison of the AUC following oral dosing with that obtained following an intravenous dose provided an estimate of the bioavailability ( $F$ ).

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